JOURNAL OF AGRICULTURAL RESEARCH

VOL. 46 WASHINGTON, D. C., JANUARY 15, 1933 NO. 2

COMPARATIVE SEROLOGICAL AND PATHOLOGICAL INVESTIGATIONS OF THE FIRE-BLIGHT ORGANISM AND A PATHOGENIC, FLUORESCENT GROUP OF BACTERIA[1]

By H. R. ROSEN, *Plant Pathologist*, and W. L. BLEECKER, *Head of Department of Bacteriology and Veterinary Science, Arkansas Agricultural Experiment Station*

INTRODUCTION

One of the most vexing problems in bacteriological research is the identification of the bacterium under investigation. As long as writers continue to describe new species on the basis of a few unverified or even verified cultural, physiological, or morphological differences within a given group of bacteria, the problem is likely to remain complex and almost insolvable. On such a basis of identification and classification one is justified in considering almost every isolate in certain groups of bacteria as representing a distinct species.

The value of serological investigations as an aid in identifying and classifying various human and animal pathogenes has been recognized for about 40 years as a highly important and almost indispensable procedure. Within recent years plant pathologists have likewise utilized such tests to some extent and in a few instances have conclusively shown (*21*, *22*, *26*, *27*, *28*, *29*)[2] that important clues to the identity and relationship of various perplexing plant pathogenes can be furnished by serological investigations. In view of the extensive series of consistent results here reported, it is hoped that, aside from the information obtained concerning the bacteria under investigation, these will offer additional evidence, if any more is needed, of the value of such studies in determining relationships among bacterial plant pathogenes.

For the last six years the senior writer has been studying the highly destructive disease of pears and apples known as fire blight. Part of these studies consisted of the gathering of empirical field data relative to the prevalence and destructiveness of the disease, varietal and species behavior of hosts, the observance of overwintered cankers and blighted organs of various types, the relationship of such blighted tree parts to the location of new infections, the relationship of insect carriers and rain and wind to disease dissemination, and observance of the organs first to show signs of the disease in the early spring. During the first three years of these investigations it was assumed that when a flower, leaf, twig, or limb of the proper host showed signs of blighting and the presence of bacteria within the tissues, fire blight was present. In many instances this was abundantly confirmed by pure culture isolations, type of growth reactions of the isolates[3] on various culture media, chemical reactions, pathogenicity tests, and

[1] Received for publication Mar. 24, 1932; issued February, 1933. Research Paper No. 286, Journal Series, Arkansas Agricultural Experiment Station.

[2] Reference is made by number (italic) to Literature Cited, p. 117.

[3] The very convenient term "isolate" recently proposed by Brierley (7), is adopted because it avoids the use of the word "strain" which is often used indiscriminately to designate a genotypic or phenotypic variant, and also to designate a particular organism isolated at a given time without reference to its taxonomic position. "Isolate" is applied specifically to the latter.

morphological studies. Over 200 of such isolates were studied and the external and internal symptoms of the natural infections from which they were derived amply observed, as were the artificial infections induced by them. Yet no suspicion arose until two years ago that the maladies considered as one disease may possibly comprise two distinct diseases induced by two different species of bacteria.

DISCOVERY OF TWO DIFFERENT BACTERIAL DISEASES OF POMES IN ARKANSAS

The possibility that two bacterial diseases occur on pears and apples first presented itself in a study of the blighting of unopened pear and apple blossoms. When such blighted blossoms were found during the first few years of these investigations, the disease was attributed to frost or insect injury or to the fire-blight pathogene, and so recorded (*34*). Not until the data for several years had accumulated relative to the absence of infectious exudate at the time of the first spring blossoms (*35*, *36*) and not until it was observed that with rare exceptions fire blight appeared first on fully opened blossoms, many of which had already been pollinated and had set fruits, did it seem worth while to study thoroughly those exceptional cases in which closed blossoms of early season formation were blighted and in which bacteria, presumably *Erwinia amylovora*,[4] were present. When several pure culture isolations from closed blossoms were carefully investigated and compared with *Erwinia amylovora* as to cultural reactions, chemical tests, morphological details, and pathogenicity, it was discovered that, with the exception of certain marked differences in symptoms in artificial inoculations, pigment production on beef-infusion agar and synthetic media, there were only slight differences or none at all in by far the largest number of tests. The chemical tests included those for the production of amylase, invertase, pectinase, ammonia, nitrite, indol, hydrogen sulphide, the digestion of milk and gelatin, and acid and alkali production. Obviously, unless one were prepared to undertake an extensive series of trials on numerous culture media, any dissimilar bacterium isolated would very likely be overlooked and would be grouped with *Erwinia amylovora*.

After it was once found that bacteria isolated from early spring blossom infections on pears produced a greenish-yellow or bluish-yellow fluorescent pigment on certain nutrient media, that their period of incubation is shorter than that of *Erwinia amylovora*, and that they produce an intense blackish discoloration of tissues along veins, midribs, and laminar tissue between veins (fig. 1) considerably ahead of bacterial penetration, it was not difficult to find numerous comparable bacteria in the early part of the growing season. The four isolates later referred to as Petal, Receptacle, Lincoln, and Garber are typical of this species and represent the kind of bacteria often present in closed, blighted blossoms of the early season, the kind present in early season leaf spotting, and in the blighting of young pear fruit.

The disease, which for convenience is now designated pear and apple blast, caused by these early season isolates in contrast to that caused by *Erwinia amylovora*, is typically a cool-weather malady. As soon as warm weather sets in it is found much less frequently. In this respect, the disease is quite comparable to citrus blast and

[4] *Erwinia amylovora* and *Bacillus amylovorus* are used synonymously in this paper.

black pit, diseases caused by a bacterium very closely related to, if not indentical with, that isolated from diseased pomes, and the temperature relationships that have been found to exist (*43*) between the citrus-blast organism and the size of spots on lemon fruits suggest a similar relationship on pomaceous hosts.

Are the differences previously enumerated sufficient to indicate that the pear and apple blast disease is distinct from fire blight and that it is caused by a different pathogene? How may the numerous points of similarity be explained? In an effort to answer these questions the serological investigations reported in the present paper were undertaken.

Baumgärtel (*4*) has found that—

> As is evident from precipitation and agglutination reactions, different kinds of bacteria grown on the same nutrient medium and under conditions precisely

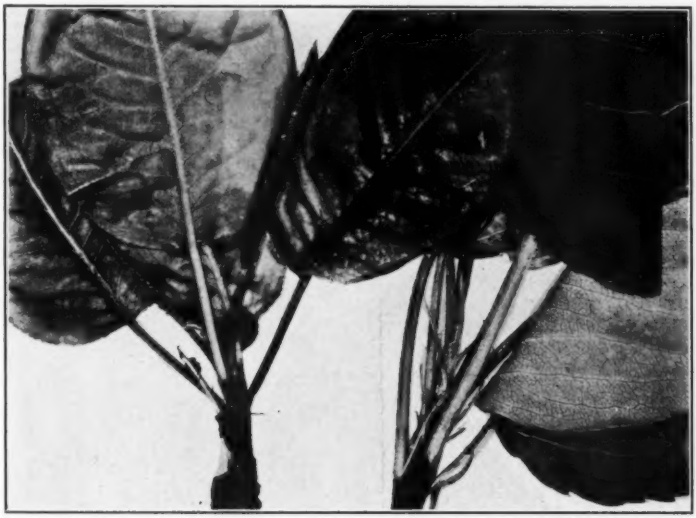


FIGURE 1.—Artificial inoculations on Bartlett pear with two of the pear-blast isolates used in the serological tests. Receptacle, right; petal, left. Photographed 20 hours after inoculation. Note the blackening of the tissues along the veins of the leaf near the center, a typical symptom of pear blast in artificial infections

similar in other respects can be differentiated with respect to their proteins, so that with the aid of the serological reactions they can be identified without further difficulty. Chemical analysis, on the other hand, offers at present no method of distinguishing the different kinds of bacterial proteins, for the methods are not sensitive enough and do not give unconditionally reliable results; nor do they permit generalization.

After it was determined that numerous isolates from pear blast (apple-blast isolates have not been so thoroughly studied up to the present) all produce a fluorescent pigment, as previously noted, a number of other fluorescent bacteria described previously as pathogenic to rosaceous plants were included in the serological tests, as well as several isolates of typical *Erwinia amylovora*. The following fluorescent species were considered: *Phytomonas syringae* (Van Hall) Bergey et al., *P. cerasi* (Griffin) Bergey et al., *P. citriputeale* (C. O.

Smith) Bergey et al., *P. papulans* (Rose) Bergey et al., *P. nectarophila* (Doidge) Bergey et al., *P. barkeri* Bergey et al., *P. prunicola* (Wormald) Wormald, and *P. utiformica* Clara.[5] A brief résumé of

FIGURE 2.—Artificial inoculations on Bartlett pear with pear-blast isolate, Receptacle (upper shoot), and with *Phytomonas syringae* on the remainder. The two middle shoots were inoculated by means of a hypodermic needle and the lower leaves by means of a spray with a bacterial suspension in sterile water on uninjured leaves. Compare with Figures 1 and 5

these fluorescent bacteria, a list of assigned host plants, and tests involving the present writers' pathogenicity studies made with some of these organisms follow.

[5] Clara calls this *Pseudomonas utiformica* sp. nov.

Cultures of *Phytomonas syringae* and *P. citriputeale* were obtained from Mary K. Bryan and compared with pear-blast isolates in morphology, cultural and physiological tests, and in pathogenicity. It was found that, while there are some differences, the organisms as a whole are a rather uniform group and that the differences between them are no greater than those between various pear-blast isolates. The symptoms produced on pear and apple by the lilac-blight organism are hardly to be distinguished from those brought about by the pear-blast isolates. (Fig. 2.) Likewise the symptoms on oranges and lemons produced by the pear-blast organism and the lilac-blight organism are directly comparable with those that have been found for the citrus blast organism. The culture of *P. citriputeale* available to the writers apparently had lost most of its virulence, as it produced no noticeable infections on orange or lemon fruit; and although 30 hypodermic injections were made into succulent Bartlett-pear shoots, only three shoots showed small more or less localized lesions as a result. The culture produced no infections on unwounded pear leaves or twigs in contrast with pear-blast isolates and the lilac-blight organism (fig. 2) which readily produced infections on unwounded tissues. From one of the lesions produced on pear by *P. citriputeale* the organism was reisolated, and when it was again inoculated into pear it caused somewhat larger spots to appear.

Wormald furnished a culture of his organism, which he named *Phytomonas prunicola*, and for the last year or more, December, 1930, to February, 1932, it has been carefully studied simultaneously with various pear-blast isolates, as well as with *P. syringae* and *P. citriputeale* (*P. cerasi* was unavailable). A very characteristic reaction of all of these is the production of a greenish-yellow, bluish-yellow, or bluish-green fluorescent pigment on beef-infusion agar and on various synthetic media, including Uschinsky's solution. The amount and quality of pigment varies somewhat with the different isolates and even with the same isolate at different times, and what one individual would class as green pigment may very readily be classed as yellow by another. As a matter of fact, the fluorescent pigment may be visualized as predominantly blue, green, or yellow, the color, aside from any true difference in strains or isolates and from the factor commonly known as the personal equation, depending on the quality and quantity of light in which the cultures are observed. Thus, while Wormald describes the pigment of his organism as yellow in Uschinsky's solution, the senior writer would call it opalescent or fluorescent greenish blue in transmitted light, and when culture tubes are held alongside of Ridgway's color charts,[6] a diffuse, reflected northern light being used, the color falls between Chrysolite green and Kildare green. The color also varies with the age of the culture. It would appear, therefore, that on the basis of pigment production it would be impractical to separate *P. prunicola* from *P. cerasi* and from the other organisms named above. As will be more fully discussed later, *P. prunicola* can hardly be distinguished from *P. syringae* and from the pear-blast pathogene either in pathogenicity tests (figs. 3 and 4) or in cultural reactions, and the serological tests further emphasize its relationship to this group.

[6] RIDGWAY, R. COLOR STANDARDS AND COLOR NOMENCLATURE. 43 p., illus. Washington, D. C., 1912.

PREVIOUS INVESTIGATIONS ON THE FLUORESCENT BACTERIA

The lilac-blight organism, *Phytomonas syringae*, first described by Van Hall in 1902 (*19*) and more recently by Bryan (*9*), is not only pathogenic to lilac but also to pear and apple, cherry (*Prunus mahaleb*), poplar (*Populus nigra*), buckwheat (*Fagopyrum esculentum*), and

FIGURE 3.—Artificial inoculations with *Phytomonas prunicola*, the English plum-wilt organism, on Bartlett-pear shoots. Compare with Figure 1

orach (*Atriplex hortensis*), as Van Hall determined in artificial inoculations. In addition to these hosts it has been found by Smith (*40*, *41*) to attack citrus, producing symptoms that are indistinguishable from those produced by the citrus blast and black-pit organism, *P. citriputeale*. In cultural characteristics the latter organism showed "a great similarity" to the lilac-blight organism. Smith likewise found a similar relationship with the pathogene concerned in gummosis and canker of stone fruits, *P. cerasi* (*41*).

With each of these three organisms, *P. syringae*, *P. citriputeale*, and *P. cerasi*, he produced artificial infections on *Prunus armeniaca*, avocado, *Chalcas exotica*, *Coprosma baueri*, *Jasminum primulinum*,

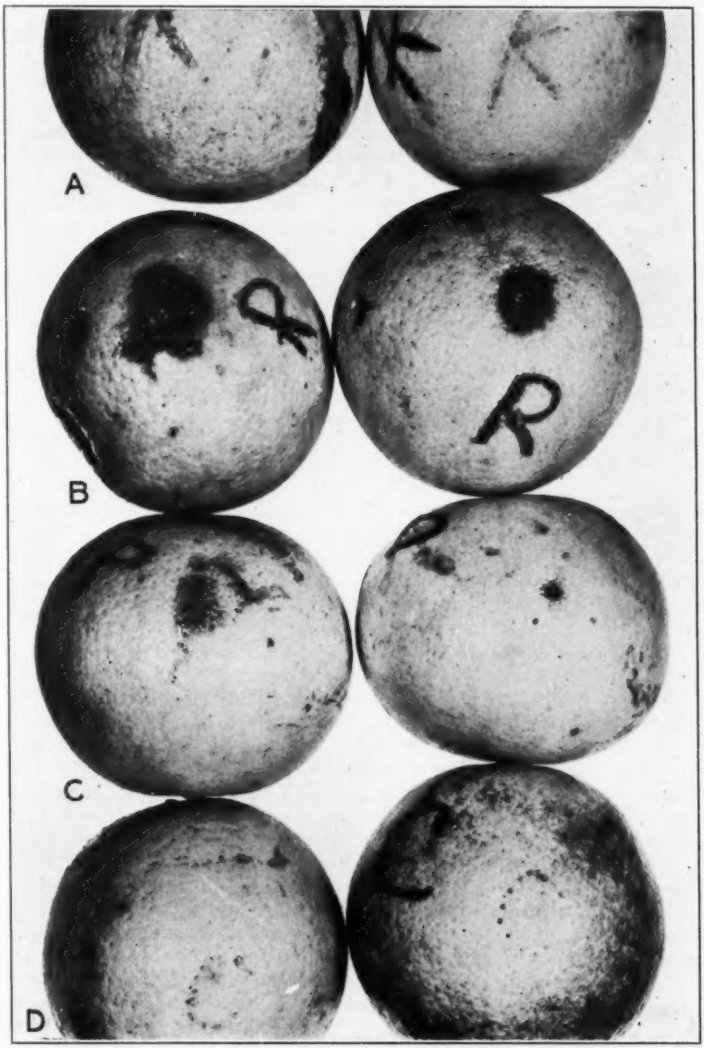


FIGURE 4.—Artificial inoculations on orange, by means of needle pricks, with pure cultures of *Erwinia amylovora* (A), pear-blast isolate, Receptacle (B), *Phytomonas prunicola* (C), and checks, sterile water (D). Photographed six days after inoculation. Note absence of infection with *Er. amylorora* and in checks, and the typical black-pit infections, characteristic of citrus blast, with pear-blast isolate and with *P. prunicola*

Fraxinus floribunda, lemons, oranges, tomato fruit, lilac, *Populus* sp., pears, and apples. Bryan (*9*) confirmed Smith's findings con-

cerning the similarity of *P. syringae* and *P. citriputeale* and decided that the two are indistinguishable. Smith, however, jointly with Fawcett (*43, p. 245*), concluded that "with our present knowledge of this group the authors would hesitate to place these three organisms * * * in a single species."

A fluorescent bacterium was described in 1917 by Rose (*31*) as the cause of a blister spot on apple fruits. He named it *Pseudomonas papulans* and presented some evidence indicating that a scurfy bark disease on apples may be due to the same or closely related organisms. In 1924, Rhoads (*30*), describing the disease known as apple measles and including under this a scurfy-bark type, concluded that it does not appear to be caused by any microorganism but is a nonparasitic (physiologic) disturbance. Hopkins [7] working in South Africa reported in 1927 a bark disease of apples consisting of blisterlike lesions which make their first appearance at the bases of the trees, whence they spread upwards and girdle the trunks. He also found the disease on young twigs and noted the similarity of bark symptoms to fire blight and to Rose's scurfy-bark disease. Several isolations from the spongy layer under the loosened epidermis gave "practically pure cultures of a bacterial organism," and comparable bacteria were isolated from other portions of the lesions. He succeeded in producing typical infections with these and in recovering the organism from the artificial inoculations. Some of the isolates produced a fluorescent pigment on certain media, while others were free from this, though he evidently decided that all the isolates represented but one species and that it was distinct from *Erwinia amylovora* and *Phytomonas papulans*.[8] Lacey and Dowson (*20, p. 36*) in 1931 described a bacterial canker of apple trees in England and concluded that the causal organism which produced a fluorescent pigment on some media "bears a certain likeness to *B. amylovorus*, but it is regarded as identical with *Ps. papulans* Rose."

In view of the doubt thrown upon the etiologic relationship of *Phytomonas papulans* by Rhoads and others, it may be desirable to record briefly that several isolations made by the senior author in October, 1924, from blackish depressed spots on Stayman Winesap apples gathered at Gravette, Ark., yielded cultures of a fluorescent bacterium that produced comparable symptoms on apple fruit when inoculated by means of needle pricks. These isolates have since been lost and will not be further considered.

A bacterial disease of pear blossoms in England was first briefly described in 1913 by Barker (*1*), and amplified by Barker and Grove (*2*) in 1914, who recorded, in addition to an attack on pear blossoms, a leaf spotting, and a dieback of spurs. Also, "there is reason to believe that a number of other plants are susceptible," including flowers of apples, cherries, gooseberries, and plums. They furth concluded that the causal organism is disseminated by bees and other insects, as they obtained the typical species of bacteria in 50 per cent of the cases when a number of bees that had been caught while working on pear blossoms were introduced on culture media in Petri dishes. They also claimed to have found the organism in the soil of fruit plantations. Cultural and morphological characters were

[7] HOPKINS, C. J. AN APPLE DISEASE OCCURING IN THE ELGIN DISTRICT. Union So. Africa Dept. Agr. Sci. Bul. 61, 17 p., illus. 1927. [Mimeographed.]

[8] Hopkins uses the terms "*Bacillus amylovorus*" and "*Pseudomonas papulans*."

presented, including the production of a greenish fluorescence on glucose-peptone media. The organism, however, was not named until 1924, when Berridge (*6*) called it *Bacillus barkeri*.

While the senior writer has been unsuccessful in producing infections with *Phytomonas barkeri* on either pear flowers or succulent twigs or leaves with two different cultures, one supplied by Barker and the other by St. John-Brooks, curator of the national collection of type cultures (Great Britain), nevertheless, there is good reason for believing that at least some of the isolates investigated by Barker and Grove represented a pathogenic species. Doidge (*12*) succeeded in producing black spots on pear receptacles with the culture that Barker and Grove sent. Wormald's (*47, p. 131–132*) brief report of a bacterial blossom wilt of pears and a bacterial fruit rot of cherries in England, with symptoms "similar to those of a disease which has been studied at the Long Ashton Research Station," indicates that a bacterial disease of pear blossoms is present in England and that it is produced by a fluorescent pigment bacterium, since Wormald reports that the isolates are closely related to his *Pseudomonas prunicola*.

In 1917 Doidge (*12*) described a bacterial blight of pears in South Africa and named the causal agent *Bacterium nectarophilum*. A comparative study of Barker and Grove's organism and the South African one was made with reference to cultural and physiological reactions and morphology. At the same time the published accounts of *Erwinia amylovora* were used as a further guide. The main differences noted between the South African organism and the English one were the presence of capsules in the former, none in the latter (Smith and Fawcett (*43*) report that citrus, Prunus, avocado, and lilac isolates are all encapsulated), the optimum temperature for growth, given as 25° to 30° C. for the South African organism and 15° to 18° in the English, and absence of gelatin liquefaction in the first and its presence in the second. (When the citrus-blast organism was grown continuously on potato-glucose agar for a year or more, Smith and Fawcett (*43*) found that its ability to liquefy gelatin is decreased or is absent.) However, the rods are comparable in size, both are polar flagellated, both produce a fluorescent pigment, though this is more noticeable in the South African pathogene, and both present similar cultural and physiological reactions on a relatively wide range of culture media. The two isolates of *Phytomonas barkeri* which the senior writer studied and which were found to be nonpathogenic under greenhouse conditions of relatively high temperatures, nevertheless grew very well at incubating temperatures of 25° to 30° C. on various culture media. As the writers have been unable to procure cultures of *Bacterium nectarophilum* from Miss Doidge, it is somewhat hazardous to make any positive statement relative to the relationship of the South African pear blossom-blight producer to other described pear pathogenes, but the fact that it belongs to the fluorescent group and possesses numerous other features characteristic of the bacterial pathogenes typified by the pear-blast organism, suggests that for the present it would be preferable to consider it a closely related variety or strain.

In 1930 Wormald (*46*) described a bacterial disease of plums involving a wilting of shoots and a spotting of leaves in natural infections. In addition to these, he also obtained a killing of buds and a

spotting of fruit in artificial infections with pure cultures. He studied the cultural characters of the pathogene carefully and compared them with the published descriptions of *Erwinia amylovora*, *Phytomonas pruni*, *P. cerasi*, and *Pseudomonas*[9] *spongiosa*. He found the group number of *Er. amylovora* as given by Stewart (*44*) to be exactly the same as that for the English plum-wilt organism, and the chief difference noted between the two was in the arrangement of flagella, that in the fire-blight pathogene being considered peritrichic and in the plum-wilt organism polar. As *P. pruni* is a yellow organism it is excluded from further consideration, and Wormald's exclusion of Aderhold and Ruhland's *Pseudomonas spongiosa* is doubtless justified because of their very brief and inadequate description. But his exclusion of *P. cerasi* because it "is stated to be a green organism" is not in accord with Griffin's (*15*) original description which reads, "* * * A chromogenic feature appears when my organism is grown on certain media, namely, a greening of the agar in plates, stabs, and slants * * *."

After the writers had completed their studies on serological reactions of *Phytomonas prunicola*, *Erwinia amylovora*, and pear-blast pathogene and had concluded that *P. prunicola* was not to be distinguished from the latter, Smith (*42*) very briefly announced that *P. prunicola*, a culture of which he had obtained from Wormald, was "apparently very similar to, if not identical with, * * * *Bact. syringae*, * * * with *Bact. citriputeale*, and with * * * *Bact. cerasi* * * *." Likewise, Wilson (*45*) in a comparison between *P. prunicola* and two different types of isolates from gummosis lesions of plum and apricot trees in California concluded that Wormald's organism resembles very closely one of his California isolates.

Shortly after the senior writer's (*37*) preliminary announcement of a new pear and apple disease in North America, simulating fire blight, and having as its etiologic agent a pathogene belonging to the group typified by *Phytomonas barkeri*, *P. nectarophila*, and *P. prunicola*, Clara (*11*) published a brief account of a new pear disease (from New York State) caused by a greenish, fluorescent bacterium. While noting that it is closely related to *P. syringae* and other fluorescent pathogenes, he nevertheless decided that it was not identical with these and was a new species, *Pseudomonas utiformica*. "* * * a comparison with the descriptions of the known species of these bacterial plant pathogenes found on pear, *Pseudomonas barkeri* and *Pseudomonas nectarophila*, showed no identity."

Despite the fact that 30 years ago Van Hall (*19*) had found that *Phytomonas syringae* is capable of attacking a number of unrelated plants including members of the Salicaceae, Polygonaceae, Chenopodiaceae, and Rosaceae, in addition to lilac (Oleaceae), his work has largely been disregarded, and species making of fluorescent pathogenes has continued unabated. This is in part explained by the fact that aside from lilac which was found naturally infected, the other hosts were only infected artificially by Van Hall. In other groups of microorganisms, such as the rusts, smuts, powdery mildews, and downy mildews, artificial infections on different hosts have become widely recognized as offering valuable criteria in determining

[9] To avoid making a new combination of a somewhat doubtful species the generic name Pseudomonas is retained, otherwise it belongs in Phytomonas.

the identity of any given fungus. Indeed such work holds exceptional interest because field behavior can be predicted by means of artificially induced infections.

Rosen and Groves (*38*), using pure cultures of *Erwinia amylovora*, were able to produce abundant infections by means of artificial inoculations on *Spiraea vanhouttei*, *Chaenomeles lagenaria* (Japanese quince), and on cultivated rose. Shortly after these results were published natural infections on *S. vanhouttei* were found in Virginia (*16*), and private reports from California and Missouri indicate that such infections were found on Japanese quince and on rose.

Among the first to investigate bacterial phytopathogenes by serological methods were St. John-Brooks, Nain, and Rhodes (*39*). They included in their studies some 13 named pathogenic species of fluorescent bacteria, including *Phytomonas barkeri* and *P. syringae*, as well as 4 nonpathogenic isolates of *Pseudomonas fluorescens* for comparison with pathogenic species. Unfortunately they made no tests for pathogenicity, so that the question may be raised as to the identity of at least some of their organisms. This is especially applicable to *P. syringae*, which in their experiments produced acid and gas in several different carbohydrate media, although several investigators, including Bryan (*9*), Smith and Fawcett (*43*), and the present writers, have noted no gas production for this species on any carbohydrate media. The production of gas by bacterial plant pathogenes is uncommon on such media. Of 93 species of such bacteria listed in Bergey's Manual (*5*), only 8 are noted as producing gas. This figure undoubtedly would be modified by closer study of the literature, but it is clearly indicative of the scarcity of gas producers among plant pathogenes. It is therefore to be questioned whether St. John-Brooks and his associates had pure cultures of *P. syringae*.

Using 16 different types of sera, including *Phytomonas syringae* and *P. barkeri* against 18 antigens, including the 2 just mentioned, at a dilution of 1 to 100, St. John-Brooks and his associates obtained with *P. syringae* serum agglutination only against the homologous antigen and against *P. atrofaciens*, while with *P. barkeri* serum agglutination was noted in the homologous antigen and in *P. tolaasi*. In view of the question raised above relative to the identity of *P. syringae* among St. John-Brooks, Nain, and Rhodes's cultures and in view of the low dilutions attempted, higher ones being contemplated for the future, one may feel somewhat doubtful as to the significance of their work on the two organisms noted, although their pioneer efforts as a whole have been extremely valuable in calling attention to the importance of serological investigations of plant pathogenes.

Goldsworthy (*14*) is the only other investigator known to the writers who has attempted serological investigation of any of the fluorescent bacteria previously listed. Having experienced difficulty in distinguishing two different bacteria[1] organisms concerned in the gummosis disease of stone fruit trees, *Phytomonas cerasi*, one of which produced a fluorescent pigment and the other did not, he decided to find out if agglutination tests would show the relationship between the two. Like other pigment producers, this one failed at times to produce pigment, so that the investigator could not tell which of the two organisms was being used at a given time. Goldsworthy found that the two would not cross agglutinate, and with homologous sera he obtained in three cases titers as high as 5,120. Had he used a larger

number of isolates, it is quite probable that he would have obtained entirely different results in the cross-agglutination tests.

METHODS USED IN THE SEROLOGICAL INVESTIGATIONS

While the methods used in agglutination and precipitation tests are becoming more and more standardized, there are sufficient variations to make it desirable to set forth the exact procedure followed. Only important points will be enumerated.

In a preliminary test a heavy bacterial suspension in 0.85 per cent saline solution of one isolate of *Erwinia amylovora*, and a similar suspension of a pear-blast isolate, 1 c c of each were injected intraperitoneally into two rabbits in order to ascertain the effect of injecting live organisms. No ill effects were noted.

To determine the agglutinating property of the normal sera of the rabbits to be used, the animals, 14 in the first series and 16 in the second, were bled from the inferior auricular vein, the blood was allowed to clot, kept in an ice chest overnight, the clot cut from the tubes and centrifuged. The supernatant sera were then added to uniform bacterial suspensions in saline solution containing 0.5 per cent phenol, in quantities to give serum dilutions ranging from 1 to 10 up to 1 to 5,120. The type of antigen used against a given serum corresponded to the one which was to be used later in immunizing the particular rabbit. All the serological tubes were then incubated overnight at 37° C. and the readings made macroscopically the following day. No agglutinations were noted in the first series. In the second series rabbits Nos. 22, 23 (Garber antigen), and 24 (*Phytomonas syringae* antigen) showed agglutination in dilutions of 1 to 10 and 1 to 20. The remainder showed no agglutination.

The density of the antigens was standardized both in the injections and in the agglutination tests by comparing the suspensions in serological tubes with a properly diluted commercial antigen of *Brucella abortus*, which had given excellent results in previous agglutination studies. The standard used in the final agglutination tests approximated the turbidity of a tube containing about 2,000,000 bacteria per cubic centimeter, while the suspensions used for injection were about fifty times as heavy.

Two rabbits were used for each isolate, and separate agglutination tests were run for each.

The culture media on which the bacteria to be injected were grown consisted of 48-hour-old slants of a commercial dehydrated potato-dextrose agar, testing pH 5.75, and to avoid the possibility of antibody reaction against the protein of potato the writers followed Link and Link's (*21*) procedure of using beef-extract dextrose-agar slants for growth of the bacteria to be used in the final agglutination tests. The latter medium was also a commercial dehydrated product, testing pH 7.2, on which the bacteria were grown for two to four days, varying somewhat with the different isolates.

Five intraperitoneal injections were made into each rabbit, the first as a 1 c c suspension and the others, three days apart, as a 2 c c bacterial suspension in each injection.

Ten days after the final injection the blood was drawn from the auricular vein of each animal. It was allowed to remain in the ice box overnight, otherwise treated as the normal blood used as the

control, and the antiserum was treated in the same manner as the normal serum.

Sufficient blood was drawn in each case to permit reciprocal tests throughout, a procedure which, as will be later shown, is very important owing to the possibility of error in some tubes, in manipulating more than 1,100 tubes in rapid order. By reciprocal tests is meant not the use of heterologous antigen against a particular antiserum, although this was of course included in the tests, but the use of a given organism as an antigen and also as an antiserum against all the other organisms included in the tests. By this method any error in manipulation is very likely to be detected. Thus, the results of the agglutination tests with Lincoln antiserum and *Phytomonas prunicola* antigen, which are included in Table 1, are to be questioned since the reciprocal with *P. prunicola* as the antiserum and Lincoln as the antigen shows entirely different results, the latter being directly in harmony with all cultural and physiological reactions of the two, as well as being in harmony with the very similar isolate, Receptacle, which shows no such discrepancy.

After any antigen was brought to its required density of suspension, it was filtered through clean glass wool in order to remove any clumps or particles of media, and in a few instances where the resulting suspension was not entirely cleared of clumps by filtration, the suspension was centrifuged and the supernatant suspension alone used in the agglutination tests.

A control tube of phenolized, saline antigen without any serum added was used in each rack and compared throughout with the tubes containing the same antigen plus antiserum. No agglutination appeared in any of the controls.

To make sure that contamination had not occurred in any of the organisms that were to be used in the serological work, and also to determine their pathogenicity at a given time, every isolate was inoculated shortly before each serological test, by means of a hypodermic needle, into three succulent pear shoots attached to growing Bartlett pear trees maintained in a greenhouse. Suitable controls in the form of hypodermic injections with sterile water were included in every trial. Every organism used in the first series was thus found to be virulent and apparently free from contaminations.

ORGANISMS USED IN FIRST SERIES OF AGGLUTINATION TESTS

As previously noted, the agglutination tests were made to determine the relationship between the fire-blight pathogene, *Erwinia amylovora*, and the pear and apple blast pathogene on the one hand, and the relationship of the pear-blast isolates to other pathogenic, fluorescent organisms on the other.

The first series included 7 different isolates, 4 typifying the nonfluorescent *Erwinia amylovora* and 3 the fluorescent pathogenes. The 4 *Er. amylovora* isolates included (1) Hlg 12/17/30, representing an isolate from honeycomb material gathered December 17, 1930; (2) Amelanchier, representing an isolate from a blighted blossom cluster of a cultivated species of Amelanchier gathered May 7, 1929; (3) Kieffer leaf spot, representing an isolate from a blighted Kieffer pear leaf gathered in June, 1931; and (4) 728, representing an isolate from a Jonathan apple twig gathered March 21, 1928. The three

fluorescent organisms included (1) Receptacle, representing an isolate from a receptacle of a Kieffer pear blossom gathered March 26, 1930; (2) Lincoln, representing an isolate from a blackened calyx cup of a young Lincoln pear fruit gathered on April 20, 1931; and (3) *Phytomonas prunicola*, representing an isolate obtained from Wormald, which he found to be the cause of a blighting of plum shoots in England. All the fire-blight and pear-blast isolates used in this series as well as in the next represent material gathered in Arkansas.

The results of the first series of serological tests are presented in Table 1. There is necessarily a great reduction in exact details, particularly in the amount of agglutination obtained in the various dilutions and in the range of dilutions utilized for each antigen antiserum.

TABLE 1.—*Titers of the first series of agglutination tests*

Type of antigen	Titer of antiserums indicated						
	Hlg. 12/17/30	Amelanchier	Kieffer leaf spot	728	Receptacle	Lincoln	*Phytomonas prunicola*
Hlg, 12/17/30	1–1, 280	1–1, 280	1–5, 120	1–2, 560	1–20	1–80	1–20
	1–5, 120	1–3, 200	1–5, 120	1–800	1–80	1–40	1–10
Amelanchier	1–3, 200	1–1, 600	1–5, 120	1–5, 120	1–40	0	1–20
	1–5, 120	1–5, 120	1–5, 120	1–5, 120	0	0	1–10
Kieffer leaf spot	1–3, 200	1–640	1–5, 120	1–2, 560	0	1–40	1–20
	1–3, 200	1–800	1–5, 120	1–1, 280	0	0	0
728	1–1, 600	1–2, 560	1–1, 600	1–1, 600	0	0	1–80
	1–5, 120	1–1, 600	1–1, 280	1–2, 560	0	0	0
Receptacle	1–10	0	0	1–40	1–160	1–1, 600	1–1, 280
	0	0	0	1–20	1–1, 280	1–2, 560	1–1, 280
Lincoln	1–40	1–40	0	1–20	1–80	1–640	1–800
	1–40	0	0	1–20	1–800	1–800	1–640
P. prunicola	0	0	0	1–10	1–80	[a]1–10	1–640
	0	0	0	0	1–320	[a]1–10	1–640

[a] The test with *P. prunicola* as antigen and Lincoln as antiserum is probably an error in final manipulation; see the reciprocal, with *P. prunicola* as antiserum and Lincoln as antigen.

Table 1 shows that the 4 isolates of *Erwinia amylovora* behave remarkably alike with respect to agglutination tests, and that the 3 isolates of fluorescent bacteria, 1 *Phytomonas prunicola*, the English plum-blight organism, and 2 isolates of American pear-blast producers, are also a homogeneous group. The 4 *Erwinia amylovora* isolates all show cross agglutination, the titers of the heterologous ones being of the same order as the homologous sera antigens. The table also shows that the *Erwinia amylovora* group present higher titers as a whole than does the fluorescent group. The same striking difference will also be noted in the later series.

It appears that, as far as these serological tests are concerned, the *Erwinia amylovora* group represents a separate and distinct species from the fluorescent group, there being very little or no cross agglutination between the two groups. Of the 48 groups of cross-agglutination tests 25 showed no reaction whatever while the remainder showed titers of such low order as to be of little significance. Serological workers in general are in agreement that titers as low as 1 to 160 are of doubtful value, being considered either as nonspecific group reactions or as indicating some process unrelated to the production of specific agglutinins.

All the tests, with the exception of the results shown in Lincoln antiserum and *Phytomonas prunicola* antigen, which are evidently an error in the final manipulation, are in agreement that *P. prunicola* is not to be distinguished from the two pear-blast isolates, Receptacle and Lincoln. This is equally evident in the four different groups of tests, whether it be where *Erwinia amylovora* isolates are used as antigens and *P. prunicola* as antisera, *Er. amylovora* as antisera and *P. prunicola* as antigens, the pear-blast isolates as antigens and *P. prunicola* as antisera, and the pear-blast isolates as antisera and *P. prunicola* as antigens. This was even more evident in the results of the final series in which five different fluorescent isolates, including *P. prunicola*, were compared. The table likewise shows that the *P. prunicola* of England and the pear-blast isolates of Arkansas produce antibodies which cross agglutinate, the titers being of the same order in homologous and heterologous tests. Thus, the antiserum of *P. prunicola* yielded even higher titers of agglutination against the pear-blast isolates than against itself.

The results in the first series show that *Erwinia amylovora* is a close, homogenous group, unrelated to the fluorescent group, and that in the latter *Phytomonas prunicola* is not to be distinguished from pear-blast isolates.

ORGANISMS USED IN SECOND SERIES OF AGGLUTINATION TESTS

A consideration of the literature previously presented clearly shows that *Phytomonas syringae*, *P. citriputeale*, *P. cerasi*, and *P. prunicola* are closely related as far as morphological, cultural, physiological, and pathological tests are concerned, although it is evident that the tests as a whole are far from exhaustive. Similar tests made by one of the writers confirm this conclusion. In addition, a number of other described "species" have been noted which apparently belong to the same group. An effort was therefore made to obtain all these organisms in order to compare them in morphological, cultural, physiological, pathological, and serological reactions. In addition to *P. prunicola*, the only other organisms that the writers were able to obtain were *P. syringae* and *P. citriputeale*. Fortunately, these three represent considerable diversity of hosts and of geographic distribution, *P. prunicola*, as already noted, coming from England and having been isolated from plum, and *P. syringae*, presumably from Illinois, with lilac as its host, and *P. citriputeale* from California, with citrus and avocado as its hosts.

In order to compare these with *Erwinia amylovora* and the pear-blast isolates from Arkansas they were similarly treated throughout, grown on various culture media, subjected to different temperatures, tested for pathogenicity (figs. 2, 3, 4, 5) on different hosts, and finally compared in serological reactions. With the exception of *Phytomonas citriputeale*, which had apparently lost its virulence, *P. syringae* and *P. prunicola* produced symptoms on pear, apple, orange, and lemon which were indistinguishable from those produced by the pear-blast isolates. (Figs. 1, 2, 3.) However, in view of the fact that the *P. citriputeale* isolate presented very comparable cultural and physiological reactions it was included in the serological tests.

As a further check on the first series of serological tests two of the isolates of *Erwinia amylovora* previously used were included,

Hlg 12/17/30 and Kieffer leaf spot, and another isolate, Jonathan limb, obtained in June, 1931, from a blighted Jonathan apple limb, was added. Two pear-blast isolates, different from those used in the first tests, were incorporated, Petal, representing an isolate from a diseased petal of a closed, blasted Kieffer pear blossom gathered

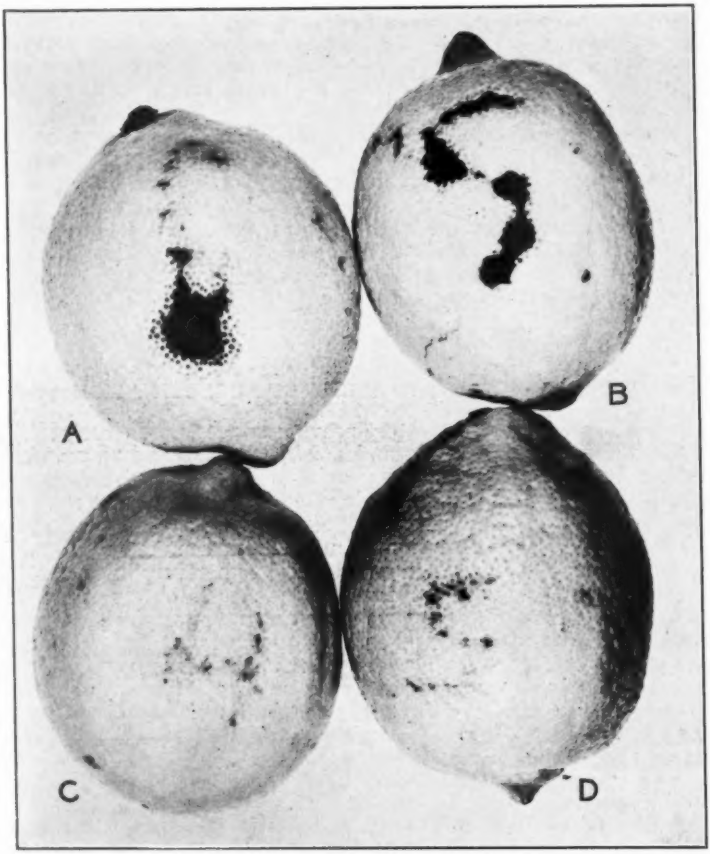


FIGURE 5.—Artificial inoculations on lemons with *Phytomonas syringae* (B); pear-blast isolate, Garber (A); *P. citriputeale* (D); and a fire-blight isolate, Hlg 12/17/30 (C). Note the infections with *P. syringae* and the pear-blast isolate, Garber, in contrast to the lack of infections with the nonvirulent *P. citriputeale* and the fire-blight isolate

March 26, 1930, and Garber, representing a young blasted fruit of Garber pear gathered April 28, 1931.

The serological tests were conducted in the same manner as previously described with the exception that four intraperitoneal injections were made instead of five. This, however, is not recommended since the titers are as a whole lower than with five injections. (Compare Table 1 with Table 2.)

TABLE 2.—*Titers of the second series of agglutination tests*

Type of antigens	Titers of antiserums indicated							
	Hlg 12/17/30	Jonathan limb	Kieffer leaf spot	Petal	Garber	*Phytomonas syringae*	*Phytomonas prunicola*	*Phytomonas citriputeale*
Hlg 12/17/30	1-5, 120	1-1, 600	1-640	0	0	0	1-40	0
	1-5, 120	1-5, 120	1-800	0	0	0	0	0
Jonathan limb	1-1, 600	1-1, 280	1-1, 280	0	1-40	0	0	0
	1-640	1-2, 560	1-800	0	1-10	1-40	0	1-20
Kieffer leaf spot	1-1, 600	1-800	1-800	0	1-10	0	0	0
	1-2, 560	1-1, 600	1-1, 600	0	0	0	0	0
Petal	1-20	1-20	0	1-160	1-320	1-320	1-320	1-800
	1-20	1-80	1-40	1-640	1-640	1-80	1-320	1-640
Garber	1-40	1-40	1-20	1-640	1-800	1-640	1-320	1-640
	1-40	1-80	1-20	1-640	1-640	1-320	1-320	1-640
P. syringae	1-20	1-80	0	1-40	1-320	1-320	1-320	1-320
	1-40	1-40	1-80	1-320	1-160	1-320	1-160	1-80
P. prunicola	1-20	1-20	0	1-320	1-160	1-320	1-320	1-320
	1-40	1-80	1-40	1-640	1-320	1-160	1-320	1-640
P. citriputeale	0	1-10	0	1-160	1-80	1-1, 280	1-640	1-800
	0	0	0	1-320	1-80	1-1, 280	1-640	1-640

Table 2 again shows the close relationship existing between the various isolates of *Erwinia amylovora*, the cross-agglutination tests showing titers as high as in the homologous sera antigens. By combining these results with those of the first series it would appear that, irrespective of the kind of host, of the kind of tissues used for isolation, or of the season of the year when the isolation is made, the isolates are so remarkably similar in these serological tests as to suggest an old firmly fixed, almost immutable species. It hardly seems possible that this will hold true for any large number of bacterial plant pathogenes, and it is to be noted that all the isolates came from but one State.

There is no apparent relationship shown between *Erwinia amylovora* and the 5 fluorescent isolates. Of the 60 linear series of cross agglutionations between the 3 isolates of *Er. amylovora* and the 5 fluorescent ones, 32 show no agglutination whatever and the remainder show that of group titer.

On the other hand, the 5 fluorescent isolates, while evidently not so homogenous as the isolates of *Erwinia amylovora*, show a very close kinship to each other. In these serological investigations the two pear-blast isolates, Petal and Garber, are hardly to be distinguished from *Phytomonas syringae*, *P. prunicola*, and *P. citriputeale*. Neither are the latter 3 to be distinguished from each other. While the titers of these are not so high as those in the *Er. amylovora* isolates, the results are so similar throughout that their relationship can be noted in any of the tests. The heterologous sera antigens present as high or even higher titers than the homologous ones. Thus Petal antiserum at a titer of 1 to 640 agglutinates Petal, Garber, and *P. prunicola*; *P. syringae* antiserum agglutinates at 1 to 320, the highest point of its own titer, Petal, Garber, *P. prunicola*, and *P. citriputeale*; *P. prunicola* antiserum agglutinates at 1 to 320 Petal, Garber, *P. syringae*, and *P. citriputeale*; and *P. citriputeale* antiserum agglutinates Petal at a titer of 1 to 800, and Petal, Garber, and *P. prunicola* at 1 to 640. Out of 50 different linear series of agglutinations in this whole group, 5 fail to show this relationship, and as the

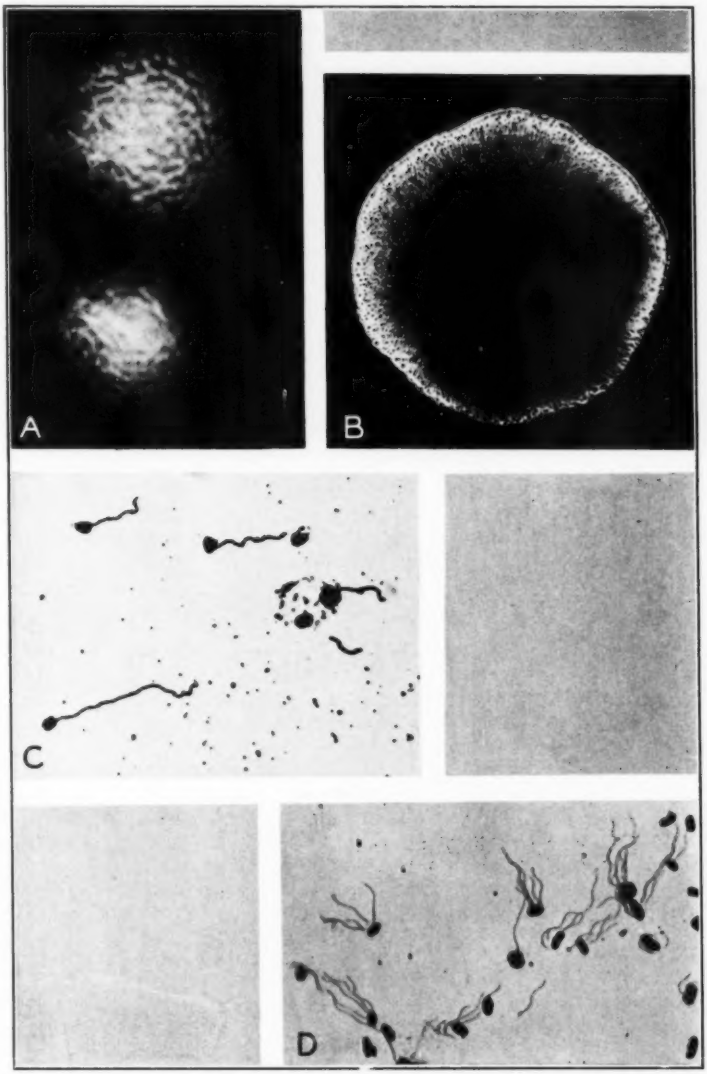
duplicates of 4 of these 5 show significant titers in the cross agglutinations the exceptions may be properly questioned. The fifth, Garber antiserum and *P. citriputeale* antigen, with two titers of only 80, are likewise to be discounted since the reciprocals run in duplicate, *P. citriputeale* antiserum and Garber antigen, present a titer of 1 to 640.

COMPARISON BETWEEN SEROLOGICAL REACTIONS, PATHOGENICITY, AND CULTURAL CHARACTERISTICS

The pathological and cultural tests, in which the senior writer, among other things, found that *Phytomonas prunicola*, *P. syringae*, and pear-blast isolates are equally pathogenic and produce similar symptoms on pears, apples, oranges, and lemons, the only hosts tried, fully confirm and amplify the serological investigations. There can be no question that from the practical point of view little or nothing is to be gained by considering them as different species. This does not preclude the possibility of some cultural, morphological, or physiological difference. They do exist in this fluorescent group to a far greater extent than in *Erwinia amylovora*. But this is not necessarily associated with any disparity in hosts. For example, the two pear-blast isolates, Receptacle and Petal, behave remarkably alike, no matter what the test may be, while the isolates, Lincoln and Garber, differ from these not only in amount and kind of pigment production but also in various other ways. Nevertheless, they were all originally isolated from pear, and all produce like symptoms in artificial inoculations. Since the difference between these is comparable to the difference in isolates on unlike hosts it appears evident that such differences can not be utilized in differentiating species within this group. To say that *P. prunicola* is a different species from *P. syringae* or from pear-blast isolates because it shows some differences would be equivalent to saying that nearly every other isolate from pear blast is a different species. Not only have variations been found in pear-blast isolates, but almost all the previous writers have noted similar differences in isolates from the same hosts in this same fluorescent group. Both Goldsworthy (*14*) and Wilson (*45*) made special studies of different isolates of *P. cerasi* and emphasized the differences in these when derived from the same host. Bryan (*9*) described a marked cultural difference between two isolates from lilac blight, one producing a rough, wrinkled colony, comparing with the feature described by Wormald (*46*) as characteristic of *P. prunicola*, and the other showing no wrinkling. She further found that agar colony characters of *P. citriputeale* were no more different from *P. syringae* than were isolates of the latter different from each other. Hopkins [10] likewise noted a marked difference in isolates from the South African bark disease of apples, some producing a fluorescent pigment and others not, and it has already been noted that Smith and Fawcett (*43*) found variation in the citrus-blast bacterium when it was grown for some time on culture media, changing from a smooth to a wrinkled growth and gradually losing its power to liquefy gelatin.

It is evident that within this group of fluorescent pathogenes there are not only disparities between different isolates from the same host but also that any one isolate may develop variations in morphology

[10] HOPKINS, C. J. Op. cit.



A, Typical colonies of bacteria, 2 days old, on beef-extract agar plates, isolated directly from a young, blasted Garber pear fruit. Transfers from individual colonies of this rough, irregular type produced typical blast symptoms on pear shoots. Compare with B. × 14. B, Typical colony of bacteria, 2 days old, on beef-extract agar, representing a reisolation from an artificial infection on pear induced by the rough, irregular type of colony originally isolated from a blasted Garber pear fruit. Compare with A. Artificial inoculations with transfers from such single, smooth, definite-margined colonies produced infections directly comparable with those produced by the rough, indefinite-margined colonies. × 14. C, *Erwinia amylorora* Hlg 12/17/30 isolate, stained to show flagella. Note the polar arrangement and compare with D. × 1,400. D, *Erwinia amylorora* Hlg 12/17/30 isolate, stained to show flagella. Note the peritrichic arrangement and compare with C. × 1,400

and in cultural (pl. 1, A and B), physiological, and pathological behavior at different times. In other words, contrasted with *Erwinia amylovora*, the fluorescent pathogenes here considered represent an elastic, highly variable group in which species delimitation on the basis of disparity in hosts or in any other recognized standard of comparison appears uncertain, and in any large measure, almost incapable of exact duplication. A carefully evolved description of any isolate or group of isolates may not be applicable to the very same isolates at another time.

The degree of infectivity as measured by the size of lesions, virulence, and loss of virulence is of interest in this group. Apparently Smith and Fawcett (*43*) hesitated to place *Phytomonas syringae*, *P. cerasi*, and *P. citriputeale* in one species (*P. nectarophila*, *P. papulans* and *P. barkeri* were not considered, and *P. prunicola* and *P. utiformica* appeared after their publication), because artificial inoculation with isolates from different hosts did not always yield comparable sized lesions on lemons, although

> the strain of *Bact. cerasi* from apricot and sweet cherry and of *Bact. syringae* from lilac when inoculated at room temperature by punctures in lemon fruits, developed typical black pit spots that were not distinguishable from those produced by *Bact. citriputeale*.

If any large number of isolates from citrus were studied alongside of an equal number from other hosts it is a question whether the citrus ones would always produce larger spots on lemons. The present writers have produced spots on both lemons and oranges at a temperature varying from 15° to 17° C. with pear-blast isolates, with *Phytomonas prunicola* and with *P. syringae* (figs. 4 and 5) which were fully as large as those found by Smith and Fawcett for *P. citriputeale*, and these isolates produced spots when *P. citriputeale*, owing to a loss of virulence, failed to produce any. If loss of virulence occurs in artificial cultures, is there any good reason for assuming that this does not occur at times in nature? Furthermore, the loss of virulence may not be complete, and the variation in virulence which has been noted by a number of investigators of this group of bacteria, notably by Barss (*3*) in *P. cerasi*, is clearly indicative of what may be expected with these fluorescent bacteria when a large number of isolates are carefully studied. As a matter of fact, when attempting to isolate a pathogene, investigators are likely to discard a culture that does not produce noticeable infections in artificial inoculations, on the supposition that it does not represent the pathogenic species. That this is not always a proper assumption has been amply shown by Patel (*29*) who found quite a number of nonvirulent strains of *P. tumefaciens* existing in nature. Burkholder (*10*) in speaking of bacterial plant pathogenes in general is obviously correct in stating that too many species have been described on the basis of pathogenicity to some host.

DISCUSSION AND CONCLUSION

The biologic reaction between a host and a given microorganism constitutes an important means of determining relationships between similar microorganisms, and it is far more specific than any commonly used cultural, physiological, or morphological test. Often, however, the reason cited for the erection of a new species is that the published accounts of some cultural or morphological characters do not fit the

organism at hand, but in only a few instances have the writers taken the trouble to verify the supposed differences.

It is to be questioned whether sufficient difference exists in either pathogenicity or in cultural and physiological reactions between *Phytomonas syringae*, *P. cerasi*, *P. citriputeale*, *P. papulans*, *P. nectarophila*, *P. barkeri*, *P. prunicola*, *P. utiformica*, and pear and apple blast isolates to justify their acceptance as distinct species. Sufficient serological evidence having been presented to show that *P. syringae*, *P. citriputeale*, *P. prunicola*, and four pear-blast isolates are alike, it would seem desirable to consider these at least as belonging to one species, and if the excellent pathogenic and cultural studies of Smith and Fawcett (*43*) on *P. cerasi* are accepted, the gummosis pathogenes would also be added. It would of course be desirable to include fluorescent pathogenes other than those already mentioned together with fluorescent saprophytes in serological studies. For the present the writers are accepting *P. syringae* as the oldest established name for this whole group of bacteria, but in view of the lack of serological evidence of a large number of other fluorescent bacteria this acceptance must be regarded as tentative. One thing seems certain with regard to these fluorescent bacteria that without serological tests the very close relationship existing between them is very likely to be underestimated. While numerous investigators have called attention to such relationship, it is questionable, judging by the array of "new species" that are continuously being described, whether its true significance has been fully realized.

From the taxonomic point of view, because of the lack of any widely accepted standards, it makes perhaps little difference whether one considers *Phytomonas syringae*, *P. cerasi*, *P. citriputeale*, *P. prunicola*, etc., as distinct species or varieties, races, strains, clons, or physiological forms of one species. The pragmatic question remains: Does the lilac-blight pathogene confine itself to lilac or does it also attack pear, apple, apricot, plum, avocado, citrus, and other hosts? If it does, and the evidence here presented substantiates this view, then what useful purpose is served in considering some isolates within this group as distinct species because of cultural or physiological disparity? When one considers the relatively large amount of evidence which is accumulating with respect to the variability or "dissociation" of bacteria, it would seem that, contrary to the rather common practice of considering a few differences as sufficient ground for separating species, such differences are to be expected in any one species. One need not accept the pleomorphism and almost endless variability theories of Löhnis (*23*, *24*, *25*), Enderlein (*13*), or Hadley (*17*, *18*) to conclude that if no differences in any group of related isolates have been found there is good reason for supposing that a given study is incomplete.

While cultural and physiological reactions in the groups of *Erwinia amylovora* and *Phytomonas syringae* show close agreement, the serological tests show practically no relationship between the two species. It has already been noted that Wormald found no difference in the group numbers between his fluorescent *P. prunicola* and *Er. amylovora*, both having exactly the same number, 211.2322033, and the chief reason given by Wormald for not considering them to be the same species is the arrangement of flagella, a character which of all bacte-

riological morphology, with the possible exception of capsules, is with the present technics apt to be most evasive and uncertain. No flagella-staining technic known to the writers has as yet been devised which has met general acceptance, and nearly every laboratory has its own particular method. The senior writer has one which has been successfully used for a number of years and which has given some excellent results (*33*), but it has not been published and probably will not be for some years because there is at present no certainty that it will give comparable results to different investigators and that it will stain the flagella of all bacterial species equally as well. This method when used on fifty-odd isolates of *Er. amylovora* shows the flagella to be variable in any one preparation, but the greatest number by far show only polar flagella (pl. 1, C), and this is consistent with the results that have previously been published (*32*). Nevertheless a few organisms can almost always be found with peritrichic flagella (pl. 1, D). By using the Casares-Gil's (Plimmer and Paine) method Bryan (*8*) finds the flagella of *Er. amylovora* only in peritrichic arrangement, and some of her excellent preparations which have been made available to the senior writer fully confirm her findings. Unfortunately, however, the writers have been unable to duplicate her work by the Casares-Gil's method. The point to note is that, irrespective of the arrangement of flagella in *Er. amylovora*, there is no flagella-staining method available at present which can always be relied on to give comparable results to different investigators. Consequently any taxonomic system which at this time is based upon either number or arrangement of flagella is likely to be insecure. The inclusion of nonmotile and polar flagellated plant pathogenes in one genus, Phytomonas, as adopted by Bergey et al. (*5*) appears to be justifiable from this point of view, but the separation of Erwinia because of flagella arrangement rests on uncertain ground. If *Bacillus carotovorus* be considered the type of Erwinia rather than *Er. amylovora*, then, with the exception of a few anomalous species which are now included, the group as a whole might be classed as gas formers in carbohydrate media, in contrast with Phytomonas, which would include all nongas formers.

While there are undoubtedly a number of cultural and physiological dissimilarities between *Erwinia amylovora* and *Phytomonas syringae*, the number of points of agreement as previously set forth is perhaps as great as those within the group of *P. syringae* itself. Were it not for the marked difference in symptoms on pears, and the total absence of infections on citrus fruit, in the case of *Er. amylovora*, it would be difficult to harmonize the cultural and physiological similarities with the serological disparities. There can be no question that the so-called salient cultural and physiological tests fail to reveal the marked difference between the two groups. On the other hand, the agreement between the pathogenicity tests and the serological reactions is perhaps the very best evidence of the reliability of serological reactions in determining relationships among bacterial plant pathogenes.

The writers conclude that the disease of pears and apples called blast is caused by the same species of bacteria that produces lilac blight, gummosis of stone fruits, citrus blast and black pit, avocado blemish, blossom blight of pears, bacterial canker of apples, and bacterial wilt of plums.

SUMMARY

Attention is directed to a disease of pears and apples hitherto unreported in North America, which simulates fire blight and which is caused by a fluorescent bacterial pathogene.

The disease, termed "blast," is noted as being most common in the early part of the growing season and as affecting closed and open flowers, leaves, and young fruits. In artificial inoculations it also involves blighting of tender twigs, with symptoms distinctly different from fire blight.

The pathogene shows, on the one hand, numerous cultural and physiological reactions identical with those of the fire-blight organism, *Erwinia amylovora*. On the other hand, it also in large measure presents characteristics that have been attributed to various fluorescent plant pathogenes, including *Phytomonas syringae*, *P. cerasi*, *P. citriputeale*, *P. papulans*, *P. nectarophila*, *P. barkeri*, *P. prunicola*, and *P. utiformica*.

A number of isolates of pear blast were compared with several *Erwinia amylovora* isolates, with *Phytomonas syringae*, *P. citriputeale*, and *P. prunicola* (the only ones available), the comparison consisting of practically all the common cultural and physiological tests, morphology, pathogenicity, and serological reactions.

This paper largely concerns itself with the serological investigations. Data concerning the pathogenicity tests are included, however, to indicate the similarity of these to the serological reactions.

Phytomonas syringae, the lilac blight organism of Europe and North America; *P. prunicola*, the English plum-wilt pathogene; and the American pear-blast isolates produce symptoms on pear twigs and leaves that are indistinguishable. These symptoms are obtainable with and without wounding. The only culture of citrus blast isolate, *P. citriputeale*, available to the writers had evidently lost its virulence. It produced no noticeable infections on lemons and oranges, though a reisolation from a very small lesion that it produced on a wounded pear twig yielded a culture which possessed a more noticeable pathogenicity for pear twigs. Pear-blast isolates, *P. syringae* and *P. prunicola*, produce symptoms on oranges and lemons that are indistinguishable from those produced by the citrus blast pathogene, *P. citriputeale*. *Erwinia amylovora* isolates, on the other hand, produced no infections on oranges and lemons.

The serological tests are found to be in full agreement with the pathogenicity studies. The pear-blast isolates show on one side no relationship to *Erwinia amylovora* and on the other side a very close kinship to *Phytomonas syringae*, *P. citriputeale*, and *P. prunicola*. Including the pear-blast isolates, these four are so closely related, as revealed in cross agglutinations, that they are indistinguishable. It is also concluded that, judging from the published accounts of *P. cerasi*, *P. papulans*, *P. nectarophila*, *P. barkeri*, and *P. utiformica*, these are probably also allied with the above and are of questionable standing as distinct species. For the present the name *Phytomonas syringae* is accepted for this group.

The significance of cultural, physiological, morphological, pathological, and serological studies in differentiation of species is discussed.

Since the current cultural and physiological tests are largely responsible for the multiplication of binomials of bacteria that are

otherwise shown to be closely related, and since the cross-inoculation tests on the different hosts involved in the fluorescent bacteria under consideration show little or no host restriction, it is contended that no useful purpose is served in erecting new species on the basis of a few cultural and physiological differences or on assumed host relationship.

The noteworthy correlation shown between pathogenicity and serological reactions is emphasized as indicative of the value of serological investigations for determining relationships of bacterial plant pathogenes.

LITERATURE CITED

(1) BARKER, B. T. P.
1913. A PRELIMINARY NOTE ON A BACTERIAL DISEASE OF FRUIT BLOSSOM AND FOLIAGE. Gard. Chron. 53:287.

(2) ——— and GROVE, O.
1914. A BACTERIAL DISEASE OF FRUIT BLOSSOM. Ann. Appl. Biol. 1:85–97.

(3) BARSS, H. P.
1915. BACTERIAL GUMMOSIS OR BACTERIAL CANKER OF CHERRIES. Oreg. Agr. Expt. Sta. Bien. Crop Pest and Hort. Rpt. (1913/14) 2:224–240, illus.

(4) BAUMGÄRTEL, T.
1928. THE CHEMICAL STRUCTURE OF BACTERIA. *In* Jordan, E. O., and Falk, I. S., ed., The Newer Knowledge of Bacteriology and Immunology, p. 14–18. Chicago.

(5) BERGEY, D. H., assisted by HARRISON, F. C., BREED, R. S., HAMMER, B. W., and HUNTOON, F. M.
1930. BERGEY'S MANUAL OF DETERMINATIVE BACTERIOLOGY; A KEY FOR THE IDENTIFICATION OF ORGANISMS OF THE CLASS SCHIZOMYCETES. Ed. 3, 589 p. Baltimore.

(6) BERRIDGE, E. M.
1924. THE INFLUENCE OF HYDROGEN-ION CONCENTRATION ON THE GROWTH OF CERTAIN BACTERIAL PLANT PARASITES AND SAPROPHYTES. Ann. Appl. Biol. 11:73–85, illus.

(7) BRIERLY, W. B.
1931. BIOLOGICAL RACES IN FUNGI AND THEIR SIGNIFICANCE IN EVOLUTION. Ann. Appl. Biol. 18:420–434.

(8) BRYAN, M. K.
1927. THE FLAGELLA OF BACILLUS AMYLOVORUS. Phytopathology 17: 405–406, illus.

(9) ———
1928. LILAC BLIGHT IN THE UNITED STATES. Jour. Agr. Research 36:225–235, illus.

(10) BURKHOLDER, W. H.
1930. THE GENUS PHYTOMONAS. Phytopathology 20:1–23.

(11) CLARA, F. M.
1932. A NEW BACTERIAL DISEASE OF PEARS. Science (n. s.) 75:111.

(12) DOIDGE, E. M.
1917. A BACTERIAL BLIGHT OF PEAR BLOSSOMS OCCURRING IN SOUTH AFRICA. Ann. Appl. Biol. 4:50–74, illus.

(13) ENDERLEIN, G.
1925. BAKTERIEN-CYCLOGENIE. PROLEGOMENA ZU UNTERSUCHUNGEN ÜBER BAU, GESCHLECHTLICHE UND UNGESCHLECHTLICHE FORTPFLANZUNG UND ENTWICKLUNG DER BAKTERIEN. 390 p., illus. Berlin.

(14) GOLDSWORTHY, M. C.
1928. THE PRODUCTION OF AGGLUTININS BY PHYTOPATHOGENIC BACTERIA. Phytopathology 18:277–288.

(15) GRIFFIN, F. L.
1911. A BACTERIAL GUMMOSIS OF CHERRIES. Science (n. s.) 34:615–616.

(16) GROVES, A. B.
1931. NATURAL FIRE-BLIGHT INFECTIONS ON SPIRAEA VANHOUTTEI. Phytopathology 21:89–91, illus.

(17) HADLEY, P.
1927. MICROBIC DISSOCIATION. THE INSTABILITY OF BACTERIAL SPECIES WITH SPECIAL REFERENCE TO ACTIVE DISSOCIATION AND TRANSMISSIBLE AUTOLYSIS. Jour. Infect. Diseases 40:1–312, illus.

(18) ——— DELVES, E., and KLIMEK, J.
1931. THE FILTRABLE FORMS OF BACTERIA: I. A FILTRABLE STAGE IN THE LIFE HISTORY OF THE SHIGA DYSENTERY BACILLUS. Jour. Infect. Diseases 48:1–159.

(19) HALL, C. J. J. VAN.
1902. BIJDRAGEN TOT DE KENNIS DER BAKTERIEELE PLANTENZIEKTEN. 198 p. Amsterdam.

(20) LACEY, M. S., and DOWSON, W. J.
1931. STUDIES IN BACTERIOSIS XVIII. A BACTERIAL CANKER OF APPLE TREES. Ann. Appl. Biol. 18:30–36, illus.

(21) LINK, G. K. K., and LINK, A. DE S.
1928. FURTHER AGGLUTINATION TESTS WITH BACTERIAL PLANT PATHOGENS. I. BACTERIUM CAMPESTRE-BACT. PHASEOLI GROUP; BACT. MEDICAGINIS VAR. PHASEOLICOLA; BACT. TUMEFACIENS. Bot. Gaz. 85:178–197.

(22) ——— and SHARP, C. G.
1927. CORRELATION OF HOST AND SEROLOGICAL SPECIFICITY OF BACTERIUM CAMPESTRE, BACT. FLACCUMFACIENS, BACT. PHASEOLI, AND BACT. PHASEOLI SOJENSE. Bot. Gaz. 83:145–160.

(23) LÖHNIS, F.
1921. STUDIES UPON THE LIFE CYCLES OF THE BACTERIA—PART I: REVIEW OF THE LITERATURE, 1838–1918. Mem. Natl. Acad. Sci. 16, 252 p., illus.

(24) ——— and SMITH, N. R.
1916. LIFE CYCLES OF THE BACTERIA. [PRELIMINARY COMMUNICATION.] Jour. Agr. Research 6:675–702, illus.

(25) ——— and SMITH, N. R.
1923. STUDIES UPON THE LIFE CYCLES OF THE BACTERIA—PART II: LIFE HISTORY OF AZOTOBACTER. Jour. Agr. Research 23:401–432, illus.

(26) MATSUMOTO, T.
1929. STUDIES ON SOME PHYTOPATHOGENIC BACTERIA WITH SPECIAL REFERENCE TO AGGLUTINATION AND COMPLEMENT FIXATION. Jour. Soc. Trop. Agr. (Nettai Nôgaku Kwaishi) 1:155–171. [In English. Japanese summary, p. 171.]

(27) ———
1930. FURTHER STUDIES ON SOME PUTREFACTIVE PHYTOPATHOGENIC BACTERIA BY AGGLUTININ ABSORPTION. Jour. Soc. Trop. Agr. (Nettai Nôgaku Kwaishi) 2:16–25. [In English. Japanese summary, p. 25.]

(28) ———
1931. ON THE CAUSAL ORGANISMS OF BACTERIAL SOFT ROT OF KOTYÔRAN, PHALAENOPSIS APHRODITE REICHB. F. Jour. Soc. Trop. Agr. (Nettai Nôgaku Kwaishi) 3:117–134. [In English. Japanese summary, p. 134.]

(29) PATEL, M. K.
1929. BIOLOGICAL STUDIES OF PSEUDOMONAS TUMEFACIENS SM. & TOWN. AND FIFTEEN RELATED NON-PATHOGENIC ORGANISMS. Iowa State Col. Jour. Sci. 3:271–298.

(30) RHOADS, A. S.
1924. APPLE MEASLES, WITH SPECIAL REFERENCE TO THE COMPARATIVE SUSCEPTIBILITY AND RESISTANCE OF APPLE VARIETIES TO THIS DISEASE IN MISSOURI. Phytopathology 14:[289]–314, illus.

(31) ROSE, D. H.
1917. BLISTER SPOT OF APPLES AND ITS RELATION TO A DISEASE OF APPLE BARK. Phytopathology 7:[198]–208, illus.

(32) ROSEN, H. R.
1926. THE NUMBER AND ARRANGEMENT OF FLAGELLA OF THE FIRE BLIGHT PATHOGEN, BACILLUS AMYLOVORUS. Mycologia 18:23–26, illus.

(33) ———
1928. VARIATIONS WITHIN A BACTERIAL SPECIES—I MORPHOLOGIC VARIATIONS. Mycologia 20:251–274, illus.

(34) ROSEN, H. R.
1928. THE RELATIONSHIP OF DIFFERENT SPECIES OF POMACEOUS HOSTS TO THE OVER-WINTERING OF THE FIRE BLIGHT GERM. Ark. Agr. Expt. Sta. (Ann. Rpt. 40) Bul. 231:68–70, illus.

(35) ———
1929. THE LIFE HISTORY OF THE FIRE BLIGHT PATHOGEN, BACILLUS AMYLOVORUS, AS RELATED TO THE MEANS OF OVERWINTERING AND DISSEMINATION. Ark. Agr. Expt. Sta. Bul. 244, 96 p., illus.

(36) ———
1930. OVERWINTERING OF THE FIRE BLIGHT PATHOGEN, BACILLUS AMYLOVORUS, WITHIN THE BEEHIVE. Science (n. s.) 72:301–302.

(37) ———
1932. TWO FORMS OF FIRE BLIGHT AND A NEW RELATED DISEASE. (Abstract) Phytopathology 22:23–24.

(38) ——— and GROVES, A. B.
1928. STUDIES ON FIRE BLIGHT: HOST RANGE. Jour. Agr. Research 37: 493–505, illus.

(39) ST. JOHN-BROOKS, R., NAIN, K., and RHODES, M.
1925. THE INVESTIGATION OF PATHOGENIC BACTERIA BY SEROLOGICAL AND BIOCHEMICAL METHODS. Jour. Path. and Bact. 28:203–209.

(40) SMITH, C. O.
1926. SIMILARITY OF BACTERIAL DISEASE OF AVOCADO, LILAC, AND CITRUS IN CALIFORNIA. (Phytopath. Note) Phytopathology 16:235–236.

(41) ———
1928. A STUDY OF CITRUS BLAST AND SOME ALLIED ORGANISMS. (Abstract) Phytopathology 18:952.

(42) ———
1931. PSEUDOMONAS PRUNICOLA AND BACTERIUM CITRIPUTEALE. Phytopathology 21:1091.

(43) ——— and FAWCETT, H. S.
1930. A COMPARATIVE STUDY OF THE CITRUS BLAST BACTERIUM AND SOME OTHER ALLIED ORGANISMS. Jour. Agr. Research 41:233–246, illus.

(44) STEWART, V. B.
1913. THE FIRE BLIGHT DISEASE IN NURSERY STOCK. N. Y. Cornell Agr. Expt. Sta. Bul. 329, p. 314–371, illus.

(45) WILSON, E. E.
1931. A COMPARISON OF PSEUDOMONAS PRUNICOLA WITH A CANKER-PRODUCING BACTERIUM OF STONE-FRUIT TREES IN CALIFORNIA. Phytopathology 21:1153–1161.

(46) WORMALD, H.
1930. BACTERIAL DISEASES OF STONE-FRUIT TREES IN BRITAIN. II. BACTERIAL SHOOT WILT OF PLUM TREES. Ann. Appl. Biol. 17: 725–744, illus.

(47) ———
1931. NOTES ON PLANT DISEASES IN 1928–1930. East Malling Research Sta. Ann. Rpt. 16th, 17th, and 18th years (1928–1930) 2 Sup.: 123–132.

CHEMICAL TRANSFORMATIONS OF PHOSPHORUS IN THE GROWING CORN PLANT, WITH RESULTS ON TWO FIRST-GENERATION CROSSES[1]

By E. E. DeTurk, *Chief in Soil Technology, Department of Agronomy, Illinois Agricultural Experiment Station;* James R. Holbert, *Senior Agronomist, Division of Cereal Crops and Diseases, Bureau of Plant Industry, United States Department of Agriculture;* and B. W. Howk, formerly *Graduate Assistant, Department of Agronomy, University of Illinois*

INTRODUCTION

In the course of cooperative corn-disease investigations carried on by the Division of Cereal Crops and Diseases of the United States Department of Agriculture and the Department of Agronomy of the University of Illinois, several inbred strains of yellow dent corn, *Zea mays indentata*, have been developed, which, after a number of generations (8 to 12) of inbreeding and selection, have become practically uniform in most visible or external characteristics, and perhaps somewhat less uniform in certain functional or internal characteristics, notably their reactions to some of the disease-producing organisms and to exposure to low and high temperatures. However, it was observed that when these inbred strains were planted on differently treated soils the responses to fertilizer treatment were distinctly lacking in uniformity among the different single-ear progenies within a given inbred line, among the different plants of many single-ear progenies, and also among certain first-generation crosses. These differences were particularly striking in the case of response to phosphate fertilizer applications. It was at once apparent that an understanding of the physiological and chemical aspects of these differences in nutritional capacity within the same species would add to the knowledge of plant metabolism, and might also contribute to the practical end of more advantageous use of fertilizers and better adaptation of corn varieties or strains to soil environment.

The work of producing the strains used and their behavior in the field will not be discussed in the present paper. This paper is concerned with a preliminary study of the distribution of various chemical forms of phosphorus in the corn plant at various stages of its development, and the distribution of the total phosphorus among four groups of compounds, organic and inorganic, in two first-generation crosses.

TRANSLOCATION AND CHEMICAL TRANSFORMATIONS OF PHOSPHORUS IN THE GROWING CORN PLANT

The vegetative period in the life of the corn plant is concerned primarily with the acquisition of inorganic substances from the soil and air, and their transformation into organic materials. During the reproductive period this process gradually gives way to a general

[1] Received for publication July 10, 1931; later withdrawn for the inclusion of additional data; issued February, 1933. This paper is an outgrowth of studies first reported in the division of agricultural and food chemistry of the American Chemical Society at its Indianapolis meeting, Apr. 1, 1931. The work reported was done in the soil fertility laboratory, Department of Agronomy, University of Illinois.

movement of these more or less elaborated materials into the developing seeds in which is concentrated at maturity a reserve of carbohydrate, protein, oil, and other organic compounds. Of the total phosphorus content of the corn plant at maturity, approximately three-fourths is to be found in the seeds; and of the portion present in the seeds, three-fourths or more occurs in the form of phytin. Values reported by Webster (*44*)[2] place the phytin phosphorus at 82.6 per cent of the total phosphorus in yellow dent corn, and 76.5 per cent in a white dent variety. Values obtained by the writers in yellow dent samples range about 83 per cent. That phytin is an important storage form of phosphorus in seeds is indicated by the relatively large amounts present in the seeds of various species. In the 11 different species reported by Webster, an average of 65 per cent of the total phosphorus was in phytin, and in only 2 species was less than 50 per cent of the total phosphorus present in this compound.

The elaboration of phosphorus compounds during the vegetative period and the translocation and storage of this element in the seeds during the reproductive stage are processes in which may be found the basis of the differential response to this element of various strains of corn such as those previously referred to. Another possible cause of the observed variations in behavior toward phosphate applications is the ability of the plants to secure the phosphorus needed for growth from the compounds present in the soil and in the soil solution. This aspect of the problem with reference to various species of plants has been emphasized in discussions of absorption by plants, or the so-called "feeding-power" of plants, by Truog (*41*, *42*, *43*), Davis, Hoagland, and Lipman (*20*), Newton (*33*), and others.

The weight of experimental evidence indicates the general absence of phytin in the vegetative parts of growing plants. Hart and Tottingham (*23*) were unable to find evidence of its presence in rutabagas or in alfalfa hay, cut in the early bloom stage. Anderson (*2*, *3*, *4*, *5*, *6*, *7*, *8*, *9*, *10*, *11*), in a series of investigations on the chemical nature of phytin, extending over about 10 years, described its isolation from seeds and seed products of many plants, but did not report its isolation from vegetative parts of plants. The work of Rather (*37*) and of Averill and King (*15*) falls in the same category with Anderson's in this respect. One exceptional case may be noted. Henrici (*24*) made an intensive study of the phosphorous compounds and their distribution in numerous grasses. These grasses were collected in South Africa, where they had grown in phosphorus-deficient soils under climatic conditions which were generally semiarid. Where the rainfall was about 18 inches annually, no phytin or water-soluble phosphorus was found in leaves of most species. At Ermelo, however, where the rainfall was 36 inches, leaves of *Eragrostis abessinica*, collected in 1926, contained 0.022 per cent of phytin P_2O_5 by the Heubner-Stadler (*26*) method, and 0.047 per cent of water-soluble P^2O^5. Young leaves of *Monocymbium ceresiiforme*, taken before jointing occurred, contained 0.061 per cent of water soluble P_2O_5, and the phytin determination gave the same value. Later in the season when seed stalks had formed, the values in old leaves had fallen to two-thirds the original values, but still remained essentially identical in the two determinations, namely, 0.039 and 0.40 per cent

[2] Reference is made by number (italic) to Literature Cited, p. 139.

for phytin P_2O_5 and water soluble P_2O_5, respectively. This is the only instance, with supporting data, which the authors have found of the presence of phytin elsewhere than in developing or mature seeds or closely associated organs, such as pericarps. Kostychev (*31, p. 215–216*), makes the following statements:

> Perhaps phytin or inosite phosphoric acid is to be regarded as the first product of the assimilation of phosphorus. * * * During the ripening of seeds, a considerable amount of phosphorus enters the seed in the form of inorganic phosphates and phytin and there are formed all the compounds of phosphorus. These are the facts concerning the transformations and movements of phosphorus compounds on the basis of thorough investigations by [W] Zaleski.

The original work of Zaleski has not been available. Scattered statements of the occurrence of this form of phosphorus in leaves or stems of plants have been seen in the early literature, but have not been accompanied by experimental data.[3]

The first experiments of the present authors were undertaken to determine the presence or absence of phytin phosphorus in different parts of growing and maturing corn plants. For this purpose, a qualitative test was considered adequate. The test in each case was carried out as follows:

The fresh plant material was macerated and then extracted with 2 per cent HCl. The filtered extract was then diluted to 0.6 per cent concentration of HCl, and $FeCl_3$ dissolved in 0.6 per cent HCl was added. The criterion used was the formation or nonformation, as a white flocculent precipitate, of the ferric salt of inosite phosphoric (phytic) acid.

Tests carried out on stalks, blades, and also on the forming tassels and ears, when present, at intervals during the prepollination period gave negative results in all cases. The first of these were made when the corn was about 3 to 4 feet high, and negative results continued until after pollination. The qualitative test indicated an abundance of phytin in developing seeds removed from the ear two weeks after pollination; but only negative results were obtained on shanks and cobs. Tests for total phosphorus on ignited samples of both shank and cob, however, gave copius precipitates of ammonium phosphomolybdate. In the case of a number of ears which were bagged, thus preventing the silks from being pollinated, tests of both shank and ear two weeks after pollination time revealed total phosphorus in abundance, but no phytin. In one bagged ear on which about a dozen scattered ovules had been fertilized, there was a small quantity of phytin in the cob outside of the few developing seeds, while within these scattered seeds, the phytin concentration was high. This was the only instance in which phytin was found in any part of the plants except developing or mature seeds.

The next experiments were carried out for the purpose of observing the enzymatic hydrolysis of phytin during the seed-germination period. Seeds of a yellow dent variety of corn were placed in a germination chamber. Aliquots consisting of 25 seeds each were withdrawn at successive 24-hour intervals, and subjected to determinations of phytin phosphorus by the Heubner-Stadler (*26*) method. The results are recorded in Table 1. The samples were exhausted at the expira-

[3] Since the preparation of the present manuscript, the paper by Knowles and Watkin (*29*) has been received, in which are reported amounts of phytin phosphorus up to 95 per cent of the total phosphorus in the vegetative parts of wheat plants which had been dried at room temperature.

tion of 144 hours. A progressive decrease in phytin is observed, which, by extrapolation of the curve, would presumably have exhausted the phytin in less than two weeks. A qualitative test for phytin made at about the same time on corn seedlings 4 weeks old, grown in soil in the greenhouse, gave negative results.

TABLE 1.—*Phytin phosphorus in germinating corn seeds after varying lengths of time in the germinating chamber*

[Total initial phosphorus in corn seeds, 0.313 per cent]

Period in moist germination chamber	Phytin phosphorus present—		Period in moist germination chamber	Phytin phosphorus present—	
	In dry corn	As percentage of the total phosphorus		In dry corn	As percentage of the total phosphorus
Hours	*Per cent*	*Per cent*	*Hours*	*Per cent*	*Per cent*
0	0.277	88.5	96	0.213	68.0
24	.264	84.3	120	[a].193	61.6
48	.264	84.3	144	[a].175	55.9
72	.254	81.1			

[a] Great difficulty was experienced in titrating in these last 2 determinations because of obscuring of the end point by soluble organic matter. (See Rather (*37*).) A method has since been devised by 2 of the authors which overcomes this difficulty and which is being published elsewhere.

These experiments indicate that phytin is not normally utilized by the corn plant as a form for transport, but is formed at or near the point of storage in the ripening of seeds, and during germination is hydrolyzed to its cleavage products, inositol, and phosphoric acid or the metallic salts of the latter, before movement into the young plant.

The accepted reversibility of enzyme reactions (see Bayliss (*16, chapter 5*)), for instance, considered with the undisputed enzymatic cleavage of phytin by phytase (*1, 8, 18, 35, 40*) leaves no doubt that phytin synthesis is an enzyme reaction. Furthermore, the fact that the beginning of this synthesis follows immediately after pollination and does not occur before pollination suggests an intimate association of activation of the zymogen with fertilization of the ovule. Inasmuch as extremely small quantities of an activator may be sufficient to set off a progressive enzyme reaction, it is not beyond the range of possibility that the pollen grain may serve as the carrier of this activator. In this connection it may be noted that Anderson and Kulp (*12*) have found that corn pollen contains nearly 1 per cent of inositol, one of the building stones of the phytin molecule, and 0.22 per cent of inorganic water-soluble phosphorous, but no phytin. The remainder of the 0.63 per cent of total phosphorus was found to consist for the most part of phosphatides.

Inasmuch as the absence of phytin from the vegetative parts of corn plants is established, steps for the separation of phytin in these parts in the analyses hereinafter reported have been omitted.

Table 2 records the total phosphorus content of individual plants of two strains of yellow dent corn grown in 1929. One of these, inbred A, is a parent of cross 365, to which reference is made later in this paper. The first-generation cross is not related to either of the two strains used in the major part of this investigation. While these data are inadequate for statistical analysis, they indicate a fairly high degree of

uniformity in phosphorus content among the individuals in a given strain when grown under similar conditions and sampled at the same stage of development.

TABLE 2.—*Total phosphorus content (per cent) in tops and roots of an inbred (inbred A) and a first-generation cross, just before pollination, grown on superphosphate-treated soil, Shirley, Ill., 1929*

Strain and plant No.	Percentage phosphorus in—		Ratio of percentage P in tops to percentage P in roots	Strain and plant No.	Percentage phosphorus in—		Ratio of percentage P in tops to percentage P in roots
	Tops	Roots			Tops	Roots	
Inbred A:				First generation cross:			
1	0.335	0.181	1.85	4	0.270	0.206	1.31
2	.330	[a].188	1.77	5	.260	.197	1.32
2a	.338			6	.274	.203	1.35
3	.322	[a].179	1.82	7	.268	.184	1.46
3a	.331			8	.268	.191	1.40
Mean	.331	.183		Mean	.268	.196	

[a] Composited sample of 2 plants.

Table 3 gives the total phosphorus content of different parts of the plants of two first-generation crosses used in this study at three stages during the season of 1930. These figures indicate an increasingly rapid movement of phosphorus from roots to tops as compared to the rate of absorption from the soil, with the approach of the reproductive stage. They also show a rapid accumulation of phosphorus in the ear at the expense of that in the vegetative parts up to two weeks after pollination, the time at which the last samples were taken.

TABLE 3.—*Total phosphorus (per cent) in different parts of corn plants of two first-generation crosses, at three stages in development, grown on untreated, phosphorus-deficient soil near Urbana, Ill., 1930*

Strain and date of sampling and stage of growth	Percentage of total phosphorus in—				Strain and date of sampling and stage of growth	Percentage of total phosphorus in—			
	Root	Top	Shank	Ear		Root	Top	Shank	Ear
F_1 365:					F_1 680:				
July 23	0.230	0.227			July 23	0.165	0.230		
Aug. 2 (pollination)	.087	.228			Aug. 2 (pollination)	.106	.258		
Aug. 16	.061	[a].150	0.341	0.495	Aug. 16	.094	[a].191	0.288	0.479

[a] Ear and shank removed at the last sampling; entire tops included in earlier samples.

Throughout the period of rapid growth during the five weeks or so preceding pollination, a gradual decline in phosphorus concentration takes place in the tops, as shown by the 1931 analyses reported in Table 4. This decline is a result of the production of plant tissue at a greater rate during this time than the rate of uptake of phosphates from the soil. Toward pollination time, phosphorus migration into the developing, but yet seedless, ear gets under way. This movement continues, with a resulting high concentration in the ears in the samples taken 10 days after pollination. At this stage there is an upward gradient from the roots through the stalks and the shanks to the ears. (Table 3.) This high concentration in the ear is manifested in a number of samples representing different strains and con-

ditions of growth, as shown in Table 5, in which an average of 0.5 per cent is present. Mature corn seeds in comparison contain only approximately 0.3 per cent. (Table 1.) It is significant that the height of phosphorus concentration is reached at a time when cellular development is at the maximum, and in advance of the maximum accumulation of organic reserves. Later this concentration is lowered by dilution as nonphosphorized materials are laid down in the endosperm. These observations are in harmony with the finding of Cockefair (*17*) that phosphorus is concentrated to the greatest extent in the regions of greatest metabolic activity. This concentration of phosphorus in the partially formed seeds soon after pollination, but before the laying down of starch is far advanced, is of interest in the light of Cockefair's suggestion of the functioning of phosphorus in the synthesis of starch.

TABLE 4.—*Trend, at successive stages of development, of total phosphorus concentration (per cent) in the aboveground portion of corn plants of eight different strains grown on phosphorus-deficient soil, with and without superphosphate fertilizer, near Urbana, Ill., planted June 7, 1931*

NO FERTILIZER

Designation of strain	Total phosphorus in samples collected on—				
	July 6	July 18	Aug. 1	Aug. 11 [a]	Aug. 22
1	0.304	0.284	0.225	0.180	0.177
2(F_1 365)	[b].322	[b].284	[b].252	[b].203	[b].192
3	.313	.276	.225	.154	.191
4	.296	.332	.253	.221	.204
5	.341	.334	.240	.182	.194
6	.376	.368	.259	.230	.179
7(F_1 680)	[b].408	[b].370	[b].279	[b].212	[b].153
8	.404	.292	.252	.241	.186

SUPERPHOSPHATE

1	0.365	0.349	0.268	0.206	0.174
2(F_1 365)	[b].346	[b].295	[b].278	[b].206	[b].186
3	.376	.288	.262	.203	.195
4	.403	.328	.288	.211	.221
5	.355	.292	.257	.191	.192
6	.381	.327	.264	.266	.226
7(F_1 680)	[b].422	[b].330	[b].283	[b].215	[b].181
8	.431	.297	.267	.231	.181

[a] This sample taken at about the pollination stage. This and the next set of samples, taken Aug. 22, represent tops with the shoots or young ears removed.

[b] Compare sum of fractions in these samples, Table 6.

TABLE 5.—*Total phosphorus content (per cent) of shanks and ears of eight samples of corn grown on untreated soil which was probably not phosphorus deficient, near Urbana, Ill.*

[Sampled August 13, 1929, approximately 10 days after pollination]

Sample No.	Description of corn	Total phosphorus content of—	
		Shank	Ear
P 1359–60	Cross No. 598, slightly diseased; treated with Semesan Jr	0.356	0.457
P 1361–62	Cross No. 573, grown from seed from parent plants not frosted	.286	.583
P 1363–64	Cross No. 573, grown from seed from frosted parent plants	.289	.478
P 1365–66	Cross No. 365, grown from seed removed from parent ear before maturity	.349	.454
P 1367–68	Cross No. 365, grown from seed removed at maturity from the same ear as P 1365–66.	.333	.536
P 1369–70	Cross No. 573, grown from seed removed from parent ear before maturity	.297	.478
P 1371–72	Cross No. 573, grown from seed removed at maturity from the same ear as P 1369–70.	.314	.506
P 1373–74	Double cross No. 517	.365	.497

CHEMICAL TRANSFORMATIONS OF PHOSPHORUS IN TWO FIRST-GENERATION CROSSES

DESCRIPTION OF PLANT MATERIAL

Two first-generation crosses were used in this study, cross 365 (A×L) and cross 680 (A48×A956). First-generation cross 365 had been recognized in previous field studies as exhibiting a pronounced response to phosphate treatment, indicated particularly by earlier maturity and also by increased yield. First-generation cross 680, on the other hand, had failed to respond appreciably to additions of phosphate fertilizers. The contrasting influence of phosphate fertil-

FIGURE 1.—First-generation crosses Nos. 680 (A) and 365 (B) growing on untreated soil. The corn shown in Figures 1 and 2 was planted the same day and the photographs were taken the same day

ization on the growth of these two crosses is shown in Figures 1 and 2, in which relative advancement in the stage of growth is the most outstanding difference. These crosses were grown on a phosphorus-deficient soil, both without fertilizer treatment and with the addition of 20 per cent superphosphate, applied and mixed with the soil around the hill at the rate of 250 pounds an acre just before the seed was planted.

Plant samples were taken for analysis at three different stages in 1930. Plantings were again made of these same strains [4] in 1931, in another part of the same field, on similar soil and with treatments the same as those used in 1930. In addition to sampling at the three stages represented by the 1930 samples, two other sets of samples were taken at earlier stages in the growth of the plants.

[4] The seed used in 1931 was produced by hand crossing the same parent inbreds, respectively, as were used the previous year, but after another generation of selection and selfing.

FRACTIONATION OF THE PHOSPHORUS COMPOUNDS

A procedure was worked out for separating the phosphorus in these samples into four fractions, making use, so far as possible, of methods already published, as noted in the following discussion. The frac-

FIGURE 2.—Continuation of the rows shown in Figure 1 across an adjacent plot treated with superphosphate at the rate of 250 pounds an acre. A, cross No. 680; B, cross No. 365

tions were as follows: (1) The portion soluble in absolute alcohol, consisting mostly of lecithin together with other phospholipids; (2) inorganic phosphates; (3) acid-soluble organic phosphorus, which includes hexose-phosphate esters and possibly some easily soluble nucleoproteins, and (4) acid-insoluble organic phosphorus compounds,

chiefly insoluble nucleic acids, and insoluble nucleoproteins which are not readily hydrolyzed.[5] Groups 3 and 4 would also include any soluble and insoluble phosphoproteins, respectively, which might be present. Phosphoproteins, however, are not thought to exist in plant tissue (Osborne (*34*)).

These separations are somewhat arbitrary. Nevertheless they accomplish a division into rather well-defined groups, on a basis of chemical properties, and also to some extent with respect to plant functioning.

All four of the determinations were made upon the same sample, the last fraction, insoluble organic phosphorus, being the total phosphorus in the residue left from the last extraction. Consequently, the total phosphorus as determined in the original material should equal the sum of the four fractions. Determinations of total phosphorus have been carried out on samples of the materials used for fractionation in the work here reported, as well as on a considerable number of other samples, with satisfactory agreement. The results of total phosphorus determinations in F_1 365 and F_1 680, as given in Table 4 are comparable with the sums of the fractions in Table 6.

TABLE 6.—*Percentage of phosphorus, separated into inorganic and three organic fractions in corn plants of two first-generation crosses, at five stages of development, grown on phosphorus-deficient soil, both with and without superphosphate fertilizer, near Urbana, Ill.; planted June 7, 1931*

[Samples of July 6 and 18 and Aug. 1 represent entire aerial portion; those of Aug. 11 and 22 had ears removed]

CORN GROWN ON UNTREATED SOIL

Strain of corn	Phosphorus fraction	Percentage of phosphorus in samples collected on—									
		July 6		July 18		Aug. 1		Aug. 11 [a]		Aug. 22	
		Dry plant material	Total amount	Dry plant material	Total amount	Dry plant material	Total amount	Dry plant material	Total amount	Dry plant material	Total amount
F_1 365	Alcohol soluble	0.033	10	0.035	13	0.034	15	0.017	9	0.021	12
	Acid soluble organic	.191	60	.124	47	.067	29	.064	34	.042	24
	Acid insoluble organic	.027	9	.029	11	.046	20	.043	23	.044	25
	Inorganic	.068	21	.077	29	.085	36	.063	34	.070	39
	Total	.319	100	.265	100	.232	100	.187	100	.177	100
F_1 680	Alcohol soluble	.082	20	.036	10	.032	12	.028	14	.025	14
	Acid soluble organic	.162	41	.148	41	.078	29	.050	25	.054	31
	Acid insoluble organic	.044	11	.051	14	.056	21	.048	24	.043	24
	Inorganic	.110	28	.128	35	.104	38	.074	37	.054	31
	Total	.398	100	.363	100	.270	100	.200	100	.176	100

CORN GROWN ON SUPERPHOSPHATE-TREATED SOIL

F_1 365	Alcohol soluble	0.041	12	0.035	11	0.037	14	0.032	15	0.024	13
	Acid soluble organic	.147	43	.122	38	.091	34	.051	25	.051	28
	Acid insoluble organic	.053	15	.049	15	.049	18	.041	20	.052	29
	Inorganic	.101	30	.117	36	.090	34	.082	40	.053	30
	Total	.342	100	.323	100	.267	100	.206	100	.180	100
F_1 680	Alcohol soluble	.063	15	.036	11	.041	15	.020	9	.030	17
	Acid soluble organic	.179	44	.127	40	.101	37	.051	24	.052	30
	Acid insoluble organic	.051	13	.034	11	.041	15	.042	20	.039	22
	Inorganic	.113	28	.119	38	.091	33	.100	47	.053	31
	Total	.406	100	.316	100	.274	100	.213	100	.174	100

[a] Pollination stage.

[5] All of the phosphorus in fraction 4 can be brought into solution by refluxing with the 2 per cent hydrochloric acid, leaving a phosphorus-free residue.

The procedure used in separating the phosphorus into the four fractions is as follows:

(1) Phospholipids. Place 5 g[6] of dried, finely ground material in a Soxhlet extraction thimble and extract continuously with absolute alcohol for 8 to 10 hours. Remove the Erlenmeyer flask containing the extract, add to it 10 ml of 40 per cent magnesium nitrate solution and evaporate to a sirupy consistency on the steam bath. Transfer to a hot plate and heat at 180° C. until decomposition ceases. Then place in a muffle furnace heated just below redness until nitrogen oxides cease to be evolved and the residue is white. Remove from muffle, cool, take up with dilute nitric acid, and determine phosphorus.

(2) Inorganic phosphorus. Completely dry the residue from the previous alcohol extraction. Transfer to a 500-ml Erlenmeyer flask and add 300 ml of 95 per cent alcohol containing 0.2 per cent by weight of hydrogen chloride gas. Stopper the flask tightly and allow to stand for 3 to 5 hours with occasional shaking. Then decant most of the alcohol through a filter into a beaker. Wash the residue thoroughly with more acid alcohol by decantation. Return the portion of the residue on the filter paper to the main portion in the flask and save. Make filtrate slightly alkaline with concentrated NH_4OH. The phosphates precipitate out as a light, flocculent precipitate. Let stand overnight and then decant through a filter, without washing. Dissolve the residue on the filter with dilute nitric acid, allowing the liquid to run into the original beaker which contains most of the precipitate. When the precipitate has entirely dissolved, determine phosphorus as usual. This is essentially the procedure recommended by Collison (*19*). The acid alcohol solution is sufficiently acid to extract all inorganic phosphates, but without appreciable hydrolysis of organic phosphorus compounds.

Various other methods for the determination of inorganic phosphorus have been proposed by Posternak (*36*) and others. These methods consist in the main of precipitation with ammonium molybdate in the cold with the solution only slightly acid. Collison has objected to this procedure on the ground that even a weakly acid aqueous solution of nitric acid will produce hydrolysis of some types of organic phosphorus compounds. Moreover, a large amount of proteinlike material is precipitated, making filtration difficult. The method recommended by Collison, as above outlined, is, on the other hand, very clean cut. Both his results and the authors' experience with it appear to justify its use.

(3) Soluble organic phosphorus. To the residue from the acid alcohol extraction, add 150 ml of 2 per cent hydrochloric acid. Stopper the flask and allow to stand with frequent shaking for three hours. Filter, using a Büchner funnel with suction, wash the residue thoroughly with water, and save for (4). Transfer the filtrate with washings to a beaker. Add 7 ml of 40 per cent magnesium nitrate solution and evaporate to dryness on a steam bath. Then heat on the hot plate until fumes cease to be evolved and the residue is white. It should not be necessary to use the muffle. Dissolve in dilute nitric acid and complete the phosphorus determination in the usual way. Preliminary experiments by one of the authors showed

[6] g is the abbreviation for gram or grams recently adopted by the Style Manual for United States Government printing.

that the 3-hour digestion at room temperature with the 2 per cent hydrochloric acid used in separating groups 3 and 4 brought to completion the extraction of the substances which could be removed under these conditions, inasmuch as other periods of extraction up to 12 hours gave the same results. This separation has been used by Anderson (*8*), Rather (*37*), and Averill and King (*15*). Kostychev (*31*) uses water extraction for the readily soluble fraction, presumably after making the alcohol-ether extraction for lipids, according to Schulze and Steiger (*39*), subsequently precipitating out the inorganic fraction by means of magnesia mixture. This scheme would involve the difficulties mentioned above in connection with Posternak's procedure.

(4) Acid-insoluble organic phosphorus. The residue from the 2 per cent HCl extraction is dried, oxidized by the procedure of Howk and DeTurk (*27*), and subjected to total phosphorus determination. The method used for estimating phosphorus in the various fractions after destruction of organic matter consists of solution of the ammonium phosphomolybdate precipitate in standard alkali and titration of the excess alkali by means of standard acid.

COLLECTION AND PREPARATION OF SAMPLES

Each sample was a composite consisting of 7 to 10 plants growing in hills of 2 and 3 plants each. An effort was made to select plants as far as possible at the same stage of development. The stage of development represented by the different samples necessarily varied to some extent because of the differences in this respect in the two strains on any given date, especially on the phosphate plot. In 1930, the first two samplings (July 23 and August 2) represented the entire above-ground portion of the plants. The last samples, taken August 16, had the young ears removed. In 1931, the samples of the first three sets represented the entire aboveground portion but in the fourth and fifth sets the ears had been removed.

In the first season's fractionations (1930) the samples were brought to the laboratory, the roots washed as free of soil as possible, and after the roots had been separated from the tops, the latter were cut in lengths of 8 to 12 inches. The material was placed in a steam-heated drying oven at a temperature of 80° to 90° C. Ventilation was secured by partly opening the doors, before which electric fans were placed. Drying progressed gradually, 24 to 28 hours being required for completion. These fractionations on both strains, grown on phosphated as well as on untreated soil, gave results similar to those shown in Figure 3. The outstanding feature of these curves is the marked reduction of acid-insoluble phosphorus accompanying the rise in the acid-soluble fraction, at a stage in growth at which it could scarcely be accounted for by dilution due to increase in total dry substance. This distribution of the phosphorus fractions thus suggests a breakdown and reutilization of complex phosphorus compounds, such as nucleoproteins, nucleic acids, etc., which have been laid down as essential constituents of the cell substance. The possibilities of the occurrence of such a phenomenon will be discussed in a later paragraph. The evidence of the literature, however, lends but scant support.

Before the next season's work was begun the drying ovens were reconstructed in such a way as to produce rapid circulation of air by means of a forced-draft blower, without essentially altering the work-

ing temperature, except for a lowering of about 10° C. at the start, brought about by rapid evaporation. Experiments not reported here were carried out on divided samples, half dried gradually and half dried rapidly in the rebuilt oven. The results showed marked differences in distribution of the total phosphorus among the different fractions under the two conditions, indicating the necessity of early arrest of enzyme activity if extensive chemical changes in the samples are to be avoided. It was also found that splitting the stalks permits much more rapid drying than cutting them crosswise, even in very short lengths.

RESULTS AND DISCUSSION

In 1931 results, which are given in full in Table 6 and presented graphically in Figures 4 and 5, were obtained by analysis of samples

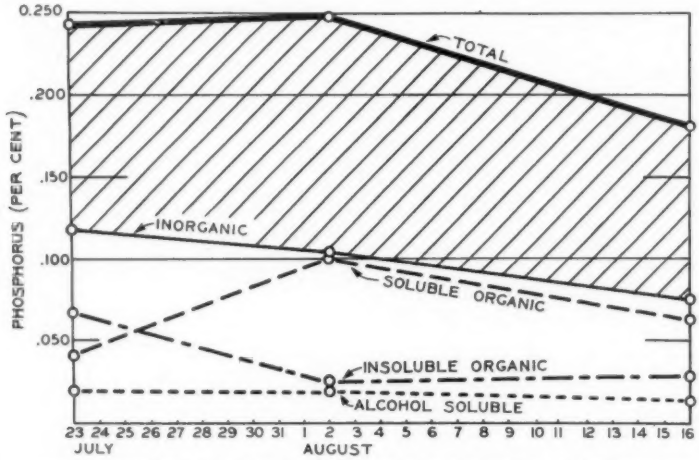


FIGURE 3.—Total phosphorus and its distribution among four fractions in a first-generation cross (F_1 680) grown in 1930 without fertilizer treatment and dried slowly in preparation for analysis

in which the stalks were quartered lengthwise and the ears cut into small pieces, the whole being placed immediately in the ovens in open wire baskets. The drying temperature during the first hour ranged from 70° to 80° C., after which it rose to 80° to 90°. The drying was accomplished rapidly, approximately 25 per cent of the total water present being removed in the first 15 minutes, and 50 to 80 per cent in the first hour. It is believed, therefore, that the rapid drying, at temperatures at which the rate of enzyme destruction or inactivation exceeds the rate of its catalytic activity, has been effective in preserving the samples essentially unchanged. (See Gortner (*22, chapter 25*) and Collatz and Bailey (*18*).)

It will be observed (fig. 4) that the four phosphorus fractions fall into two groups. On the one hand are the nucleoproteins and related compounds (insoluble organic residue) and phospholipids (alcohol-soluble substances) which comprise an approximately constant part of the plant tissue throughout the growing season. These make up the more complex forms of phosphorus, the chemical la-

bility of which is very low. They are little if at all subject to translocation after having once been laid down in the cells. If any translocation occurs, the data indicate that it is in the lecithin fraction. As some of the other forms of phosphorus move into the ear in the later stages, leaving a lowered total concentration in the vegetative parts, the ratio of insoluble organic to total phosphorus rises (Table 6) because none of this fraction has moved out. In fact, the rise begins as early as August 1, during the period of rapid plant growth before pollination, when total phosphorus concentration is being reduced through dilution by newly synthesized material, thus indicating that the rate of formation of this fraction is keeping pace with plant growth. In the case of lecithin no rise in the ratio to total phosphorus is observed. Thus there is an indication that a portion of it may be broken down for transfer into the ear. The same possibility has been suggested by Eckerson (*21*), who found that in the tomato plant under conditions of phosphorus starvation the order of disappearance from older tissues is inorganic phosphate, starch-bound phosphorus, and phospholipids. The last named did not break down until most of the starch had disappeared. She says: "Although it is not certain that this (lipid) phosphorus can be reutilized, it seems possible." Koch and Reed (*30*) in the study of Aspergillus found that in phosphorus starvation after the disappearance of all of the water-soluble inorganic and organic phosphorus, lecithin decreased, but no loss of the nucleoproteins was established. In Figure 5, A and C, the percentages of lecithin and insoluble organic phosphorus have been combined and plotted as "nonlabile" phosphorus along with the other two groups, shown as "labile" phosphorus. The minimum requirement of the vegetative plant for the nonlabile material is shown by these data (figs. 4 and 5, A and C) to be a definite concentration which must be reached regardless of the nutritional environment. The concentration is essentially the same not only at the different growth stages but also in plants grown with either a deficient or abundant phosphate supply. Phosphorus absorbed in so-called luxury consumption is evidently not converted into these forms.

The other two fractions are characterized by marked chemical reactivity. These are the inorganic phosphates and the acid-soluble organic phosphorus. In the period covered by the first three samplings, no information is furnished on phosphorus movement because each sample includes the entire aerial portion of the plants. The rapid fall in concentration of acid-soluble organic phosphorus during this period must, then, be explained by its conversion to the nonlabile forms to meet the requirements of the rapidly expanding plant. (Fig. 4.) The absorption of inorganic phosphorus from the soil keeps pace at first, as shown by the rising concentration, but at the steepest part of the growth curve [7] it falls behind, as is seen in the change to a downward slope of the inorganic curve at the second sampling period in three cases, and at the third period as shown in Figure 4, A. The continued decline in these two constituents after pollination, shown, combined, as "labile" phosphorus in Figure 5, points to them as the source of phosphatic material for deposition

[7] The general character of the growth curve is shown by the superimposed curves for two of the four cases in fig. 4, B and C.

in the maturing seeds, as well as for formation of the stable lipid and nuclein phosphorus compounds in vegetative parts.

The data of the present investigation (fig. 4 and Table 6) do not prove that the soluble organic fraction is actually transported with-

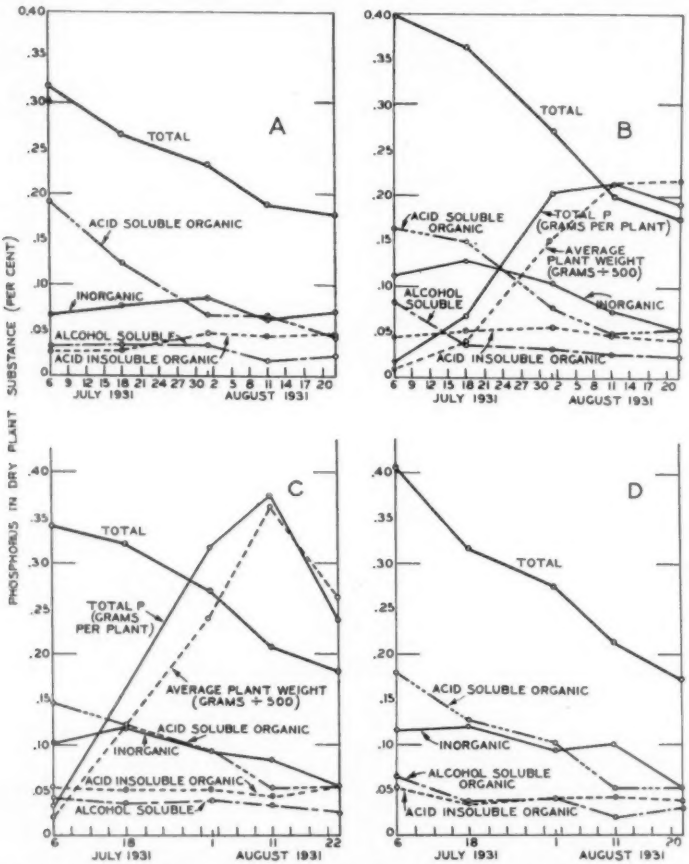


FIGURE 4.—Total phosphorus and its distribution among four fractions in two first-generation crosses of yellow dent corn. A and B give the values for F_1 365 and F_1 680, respectively, grown on phosphorus-deficient soil without fertilizer treatment; C and D give the values for the same crosses, respectively, grown on soil treated with superphosphate at the rate of 250 pounds an acre. In B and C are also shown the weights of the dry plants and the grams of total phosphorus per plant for two of the four cases. The marginal legend at the left applies also to these curves if the marginal notation be read "grams." The data are from Table 6

out previous reconversion to the inorganic state. Either could be converted to the other in connection with the translocation.

The literature furnishes satisfactory evidence of the migration of phosphorus from tissues already formed into areas of new growth. For instance, the early investigations of Arendt (*14*) and of Wilfarth, Römer, and Wimmer (*45*) are cited. The results of Koch and Reed (*30*),

Eckerson (*21*), MacGillivray (*32*), Andre (*13*), Henrici (*24, 25*), Reed (*38*), Jones and Huston (*28*), and others give further confirmation.

The question of the reutilization of phosphorus is not so easily settled. A discussion of this topic necessitates a definition of the term. As used here it will be understood to refer to the breakdown and translocation of those phosphorus-containing constituents of the cell which are essential to its functioning or to cell division. Thus nucleic acids, nucleoproteins and phospholipids will be included, represented

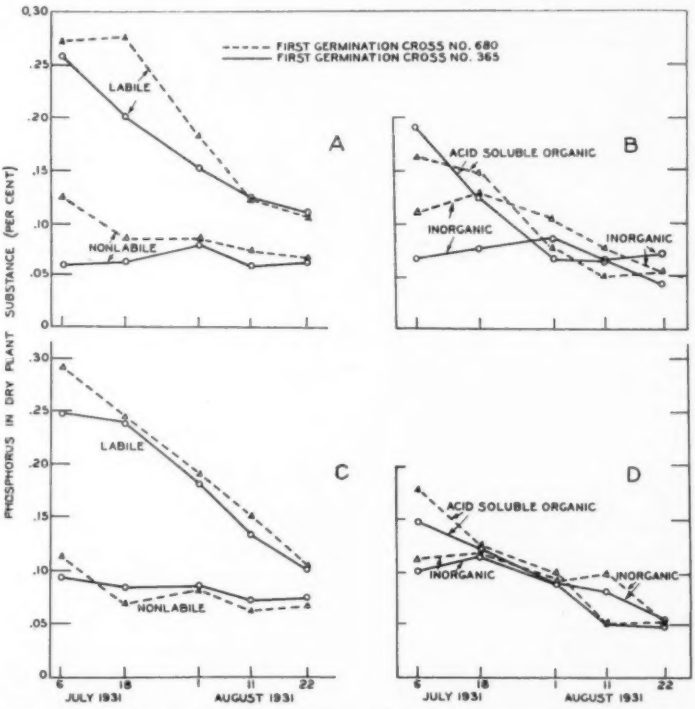


FIGURE 5.—Labile and nonlabile phosphorus in two first-generation corn crosses grown without (A) and with (C) superphosphate treatment. The labile fraction is the sum of the inorganic and the acid-soluble organic fractions. The nonlabile fraction is the sum of the alcohol-soluble and the acid-insoluble fractions. The two components of the labile fraction are shown separately for corn from untreated soil in B, and for corn from soil treated with superphosphate in D.

by groups 1 and 4 in the separations reported in this paper. The movement and use of the phosphorus-containing constituents of groups 2 and 3 including inorganic phosphates, hexose phosphates, and other soluble forms, as well as phytin, will not be considered as reutilization because these substances are not actual constituents of the living substance of the plant cell; they are merely present. Phytin is primarily a storage form and the inorganic and soluble organic compounds are of value chiefly for transport, the last-named probably constituting transition products in more complex syntheses as well.

The results of the present investigation and also a search through the literature have failed to reveal conclusive evidence of the reutilization of phosphorus. The results previously mentioned of Eckerson and of Koch and Reed with reference to phospholipids are the most suggestive but are not conclusive. MacGillivray (*32*) states in connection with his work on the tomato plant that "there is a reutilization of the phosphorus," and quotes André (*13*) as having shown a similar phenomenon. The phosphorus in MacGillivray's tomato plants, which was first carried into the lower leaves and later transferred to other parts may have consisted entirly of soluble compounds which had not become vital constituents of the cells of the lower leaves, whither they had first been transported. A classification of the phosphorus compounds would have been necessary in order to answer the question in this case. The facts shown by André were (1) that 100 beans contained 0.9828 g of total P_2O_5 and (2) that in 100 plants approximately 30 cm long grown from similar seeds without nutrient salts, 26 per cent of the original 0.9828 g of P_2O_5 was in the cotyledons and 67.3 per cent in the roots and tops, leaving 6.7 per cent unaccounted for. Webster's (*44*) analyses include several different kinds of seeds in which more than 74 per cent of the phosphorus is in phytin and inorganic combinations; i. e., storage and portable forms, not counting soluble organic phosphorus which he did not determine. No data on fractionations of phosphorus in garden beans have been found. In the absence of such data, and in view of the large amounts of transport and storage forms in seeds as shown by Webster, one would scarcely be justified in assuming that a breakdown and reutilization of the phosphorus of the protoplasm had occurred in André's experiment. The analyses of Henrici (*24*, *25*) are of special interest in relation to this question. She carried out total phosphorus determinations at weekly, or in some cases fortnightly, intervals on the roots, crowns, leaves, jointed stems, and heads of 16 species of veld grasses and of some other plants found growing in the phosphorus-poor soils of South Africa near Pretoria. The analyses were continued for 12 to 13 months and parts of two other seasons. The yearly rainfall, varying from about 18 to 36 inches, was a limiting factor of growth in most cases. In another paper (*24*) she reported results of phosphorus fractionations on some of these grasses into essentially the same groups as those determined in this investigation. She found that in the regions of lowest rainfall, soluble phosphorus was absent or present in extremely small quantities in the leaves of most grasses. At Vryburg, with 18 inches of rain, no inorganic phosphorus was present in collective grass samples. In these cases the formation of heads was not accompanied by a decline in concentration of leaf phosphorus. The same was true at Armoedsvlakte with the same rainfall. Few species of the perennial grasses produced sound seeds under these conditions, and some produced no jointed stems. Two annual weeds, *Tragus* sp. and *Aristida* sp. growing in the same region always produced an abundance of viable seeds. Their leaves carried about the same percentage of total phosphorus as those of the grasses, but the heads reached 0.8 per cent. The practically seedless heads of grasses scarcely exceeded the low concentrations of the leaves (up to 0.3 per cent). Another characteristic plant of this semiarid region, *Salsola zeyheri*, always contained inorganic phosphorus in the leaves, produced seed abundantly, and seed

formation was accompanied by a decline in phosphorus concentration in the leaves.

Phosphate absorption by plants in that region is limited apparently by the water shortage as well as by the small amounts of phosphorus present in the soil. In the case of *Digitaria eriantha*, growing with 22 inches of rain near Vryburg, abundant stalks and heads but no seeds were formed; with 19 inches, comparatively few heads appeared; and with 8½ inches only an occasional stem was found. At Ermelo, with 36 inches annual rainfall, another species of Digitaria produced abundant seeds, and the process was accompanied by decline in leaf phosphorus. The author does not state, however, whether soluble phosphorus was present in the leaves in this case. These results afford a strong indication that in the numerous species studied, the production of seeds depends largely upon whether there is more phosphorus available than is required for the organic cell constituents of the vegetative parts; and they also point to the inability of the plants to reutilize such organic phosphorus compounds, once they are built into the cell structure.

Comparison of the Two First-Generation Crosses

TOTAL PHOSPHORUS

The total phosphorus data for the two strains may be compared in Figure 4. On untreated soil, the nonresponsive cross, 680, maintained a definitely higher concentration than cross 365 except at the last sampling. Computing from Table 6, the increases or decreases (−), in concentration of total phosphorus in No. 680 over No. 365 are, for the five periods, respectively, 25, 37, 16, 7, and −1 per cent. These figures evidently represent greater absorption of phosphorus rather than higher percentages due to less growth. Figure 1 is a typical representation of the two crosses growing on untreated soil shortly before the third sampling period. Photographs taken in three different years reveal the same situation. It will be observed that the average plant heights are about the same for the two crosses. Cross 365 is somewhat stockier than cross 680 and would probably be a little heavier. Unfortunately the data on plant weights and amounts of phosphorus per plant are incomplete. The available evidence would seem to indicate that cross 680 has greater ability to absorb phosphate from a limited supply. When, however, the supply is made adequate this cross fails to maintain a higher absorption rate. The increases or decreases (−) of total phosphorus, in No. 680 as compared to No. 365, computed as above, are for the five successive samplings 19, −2, 3, 3, −3 per cent. Under these conditions, with its greater growth (fig. 2) No. 365 has unquestionably taken up more phosphorus from the soil than has No. 680. Whether the greater growth is the result of more rapid phosphate absorption or whether the greater absorption is the result of the greater growth and more extensive root system of No. 365 can not be stated.

PHOSPHORUS FRACTIONS

Some of the results of the analyses of the two first-generation crosses are brought together for comparison in Figure 5. The supplying of abundant phosphate largely masks differences in the composition of the two strains, as may be seen in the close parallelism of the curves

for the superphosphate plots. (Fig. 5, C and D.) More pronounced differences are to be observed in the plants grown with limited available phosphate. The level of the nonlabile or protoplasmic phosphorus does not differ greatly in the two crosses, regardless of supply. However, with limited supply, No. 680, the nonresponsive cross, has a slightly higher level. It will be noted also that the two strains apparently reverse positions in this respect with the addition of abundant phosphate. It can not be stated that these small differences are outside the range of experimental error, but they are consistent throughout most of the period covered by the samplings. The chief differences between the two strains are to be found in the more labile fractions in plants which have been grown with inadequate phosphate. The nonresponsive cross, No. 680, contains a distinctly higher concentration of inorganic phosphorus during active vegetative growth. The difference gradually diminishes and disappears soon after pollination. (Fig. 5.) This is the only respect in which the two crosses deviate from each other appreciably in phosphorus composition. It can not be said that this difference is due to slower conversion of inorganic phosphorus to the various organic forms, for the variations in all three organic fractions, while they are too small to merit serious consideration, tend in the direction of higher concentrations in cross 680. This, in itself, is suggestive of a possible higher minimum requirement of phosphorus for the maintenance and functioning of the vegetative machinery in this cross as compared to the more responsive one.

SUMMARY AND CONCLUSIONS

It has been found in the case of open-pollinated yellow dent corn grown in the field that phytin is absent from all parts of the plant before pollination.

Within two weeks after pollination phytin appears in the developing seeds, but is not present in other parts of the plant. At this stage of growth there is an increasing gradient in total phosphorus concentration from the roots through the stalk and shank to the ear. These facts indicate a close association of the phytin-forming function with fertilization of the ovule, and the possibility is suggested that the activator of the zymogen of the enzyme responsible for phytin synthesis may be furnished by the pollen.

Phytin disappears from corn seeds at a fairly rapid rate during germination.

The phosphorus in the tops of two first-generation crosses, grown in the field in phosphorus-deficient soil, both with and without superphosphate fertilizer, was separated into one inorganic and three organic fractions at five stages of growth. Three of these stages were before pollination, one approximately at pollination, and one 11 days later. The two crosses differed in their response to phosphate fertilizers both in growth and in yield. The phosphorus fractions separated were: (1) Absolute alcohol extract (phospholipids); (2) inorganic; (3) organic, soluble in cold aqueous 2 per cent HCl; and (4) organic residue insoluble in this HCl (nucleic acids and nucleoproteins).

Two fractions, phospholipids and acid-insoluble organic phosphorus, remained fairly constant in percentage of the dry matter throughout the period of the experiment.

The other two fractions exhibited marked variations in concentration at successive samplings, the trend being toward a decrease in acid-soluble organic phosphorus throughout the duration of the experiment, and a progressive decrease in the inorganic fraction after the second sampling.

It is concluded as a result of this investigation that phosphorus translocated from vegetative parts of the plant into the ears consists only of these two labile fractions, namely, inorganic and acid-soluble organic compounds, with the possible exception of a breakdown of small amounts of phospholipids.

As a result of this work and a study of the literature, it is considered very doubtful whether phosphorus which has been laid down in essential organic constituents of plant cells can be transported and reutilized.

This investigation has not so far revealed a satisfactory explanation of the mechanism for the differential behavior of the two crosses studied with respect to phosphate fertilization.

LITERATURE CITED

(1) Adler, L.
1916. Gewinnung von phytase aus malz. Biochem. Ztschr. 75: [319]–338.

(2) Anderson, R. J.
1912. Phytin and phosphoric acid esters of inosite. N. Y. State Agr. Expt. Sta. Tech. Bul. 19, 17 p.

(3) ———
1912. Phytin and phosphoric acid esters of inosite. II. N. Y. State Agr. Expt. Sta. Tech. Bul. 21, 16 p.

(4) ———
1912. The organic-phosphoric acid compound of wheat bran: preliminary report. (third paper on phytin.) N. Y. State Agr. Expt. Sta. Tech. Bul. 22, 16 p.

(5) ———
1912. The organic phosphoric acid of cottonseed meal. N. Y. State Agr. Expt. Sta. Tech. Bul. 25, 12 p.

(6) ———
1914. A contribution to the chemistry of phytin. N. Y. State Agr. Expt. Sta. Tech. Bul. 32, 44 p.

(7) ———
1914. Organic phosphoric acids of wheat bran. N. Y. State Agr. Expt. Sta. Tech. Bul. 36, 20 p.

(8) ———
1915. Concerning the organic phosphorus compound of wheat bran and the hydrolysis of phytin. N. Y. State Agr. Expt. Sta. Tech. Bul. 40, 31 p.

(9) ———
1920. Concerning inosite phosphoric acids. N. Y. State Agr. Expt. Sta. Tech. Bul. 79, 22 p.

(10) ——— and Bosworth, A. W.
1916. Concerning the utilization of inosite in the animal organism. I. Concerning the effect of inosite upon the respiratory exchange in the dog. . . . II. The effect of inosite upon the metabolism of man. N. Y. State Agr. Expt. Sta. Tech. Bul. 54, 16 p.

(11) ——— with the collaboration of Kulp, W. L.
1921. An investigation of the seed of the silver maple (acer saccharinum). N. Y. State Agr. Expt. Sta. Tech. Bul. 81, 20 p.

(12) ——— and Kulp, W. L.
1922. Analysis and composition of corn pollen. Preliminary report. Jour. Biol. Chem. 50:433–453.

(13) André, G.
1918. Répartition des éléments minéraux et de l'azote chez le végétal étiolé. Compt. Rend. Acad. Sci. [Paris] 167:1004–1006.

(14) Arendt, R.
1859. untersuchung über einige vorgänge bei der vegetation der haferpflanze. Landw. Vers. Sta. 1:31–66.
(15) Averill, H. P., and King, C. G.
1926. the phytin content of foodstuffs. Jour. Amer. Chem. Soc. 48:724–728.
(16) Bayliss, W. M.
1925. the nature of enzyme action. Ed. 5, 200 p., illus. London, New York.
(17) Cockefair, E. A.
1931. the role of phosphorus in the metabolism of plants. Amer. Jour. Bot. 18:582–597.
(18) Collatz, F. A., and Bailey, C. H.
1921. the activity of phytase as determined by the specific conductivity of phytin-phytase solutions. Jour. Indus. and Engin. Chem. 13:317–318, illus.
(19) Collison, R. C.
1912. inorganic phosphorus in plant substances. an improved method of estimation. Jour. Biol. Chem. 12:65–72.
(20) Davis, A. R., Hoagland, D. R., and Lipman, C. B.
1923. the feeding power of plants. Science (n. s.) 57:299–301.
(21) Eckerson, S. H.
1931. influence of phosphorus deficiency on metabolism of the tomato (lycopersicum esculentum mill). Contrib. Boyce Thompson Inst. Plant Research 3:197–217, illus.
(22) Gortner, R. A.
1929. outlines of biochemistry; the organic chemistry and the physicochemical reactions of biologically important compounds and systems. 793 p., illus. New York and London.
(23) Hart, E. B., and Tottingham, W. E.
1909. the nature of the acid soluble phosphorus compounds of some important feeding materials. Jour. Biol. Chem. 6:431–444.
(24) Henrici, M.
1927. phosphormangel als ursache von störungen im leben der pflanze. Verhandl. Naturf. Gesell. Basel 38:[316]–326.
(25) ———
1928. the phosphorus content of the grasses of bechuanaland in the course of their development. Union So. Africa Dept. Agr. Dir. Vet. Ed. and Research Rpts. 13 and 14 (pt. 2):[1077]–1208, illus.
(26) Heubner, W., and Stadler, H.
1914. über eine titrationsmethode zur bestimmung des phytins. Biochem. Ztschr. 64:[422]–437, illus.
(27) Howk, B. W., and DeTurk, E. E.
1932. ashing plant materials to determine total phosphorus. Indus. and Engin. Chem., Analyt. Ed. 4:111–112.
(28) Jones, W. J., jr., and Huston, H. A.
1914. composition of maize at various stages of its growth. Ind. Agr. Expt. Sta. Bul. 175, p. [599]–630, illus.
(29) Knowles, F., and Watkin, J. E.
1932. the amounts and distribution of some phosphorus and nitrogen compounds in wheat during growth. Jour. Agr. Sci. [England] 22:755–766.
(30) Koch, W., and Reed, H. S.
1907. the relation of extractive to protein phosphorus in aspergillus niger. Jour. Biol. Chem. 3:49–52.
(31) Kostychev, S.
[1931]. kostychev's chemical plant physiology. . . . Transl. and ed. by C. J. Lyon. 497 p., illus. Philadelphia.
(32) MacGillivray, J. H.
1927. effect of phosphorus on the composition of the tomato plant. Jour. Agr. Research 34:97–127, illus.

(33) NEWTON, J. D.
1923. A COMPARISON OF THE ABSORPTION OF INORGANIC ELEMENTS, AND OF THE BUFFER SYSTEMS OF LEGUMES AND NON-LEGUMES, AND ITS BEARING UPON EXISTING THEORIES. Soil Sci. 15:181–204, illus.
(34) OSBORNE, T. B.
1919. THE VEGETABLE PROTEINS. 125 p. London, New York, [etc.].
35) PLIMMER, R. H. A.
1913. THE METABOLISM OF ORGANIC PHOSPHORUS COMPOUNDS. THEIR HYDROLYSIS BY ENZYMES. Biochem. Jour. 7:43–71.
(36) POSTERNAK, S.
1919. SUR LA CONSTITUTION DU PRINCIPE PHOSPHO-ORGANIQUE DE RÉSERVE DES PLANTES VERTES. Compt. Rend. Acad. Sci. [Paris] 169:37–39.
(37) RATHER, J. B.
1917. THE DETERMINATION OF PHYTIN PHOSPHORUS IN PLANT PRODUCTS. Jour. Amer. Chem. Soc. 39:2506–2515.
(38) REED, H. S.
1907. THE VALUE OF CERTAIN NUTRITIVE ELEMENTS TO THE PLANT CELL. Ann. Bot. [London] 21:[501]–543, illus.
(39) SCHULZE, E., and STEIGER, E.
1889. ÜBER DEN LECITHINGEHALT DER PFLANZENSAMEN. Hoppe-Seyler's Ztschr. Physiol. Chem. 13:[365]–384.
(40) SUZUKI, U., YOSHIMURA, K., and TAKAISHI, M.
1907. ÜBER EIN ENZYM "PHYTASE" DAS "ANHYDRO-OXY-METHYLEN DIPHOSPHORSÄURE" SPALTET. Tokyo Imp. Univ. Col. Agr. Bul. 7:[503]–512. [Original not seen.]
(41) TRUOG, E.
1916. THE UTILIZATION OF PHOSPHATES BY AGRICULTURAL CROPS, INCLUDING A NEW THEORY REGARDING THE FEEDING POWER OF PLANTS. Wis. Agr. Expt. Sta. Research Bul. 41, 50 p., illus.
(42) ———
1922. THE FEEDING POWER OF PLANTS. Science (n. s.) 56:294–298.
(43) ———
1928. HOW PLANTS FEED. First Internatl. Cong. Soil Sci. Proc. and Papers (1927) 3:628–636.
(44) WEBSTER, J. E.
1928. PHOSPHORUS DISTRIBUTION IN GRAINS. Jour. Agr. Research 37:123–125.
(45) WILFARTH, H., RÖMER, H., and WIMMER, G.
1906. ÜBER DIE NÄHRSTOFFAUFNAHME DER PFLANZEN IN VERSCHIEDENEN ZEITEN IHRES WACHSTUMS. Landw. Vers. Sta. 63:1–70, illus.

THE BIOLOGY OF OPIUS MELLEUS GAHAN, A PARASITE OF THE BLUEBERRY MAGGOT[1]

By F. H. LATHROP, *Senior Entomologist, Division of Deciduous Fruit Insects,* and R. C. NEWTON, *Junior Entomologist, Division of Cereal and Forage Insects, Bureau of Entomology, United States Department of Agriculture*

INTRODUCTION

One of the interesting phases of the investigation of the blueberry maggot, *Rhagoletis pomonella* Walsh,[2] in eastern Maine (*7*)[3] proved to be a study of the biology of the parasite *Opius melleus* Gahan.[4, 5] The study reported in this paper was conducted at Cherryfield, Me., during the years 1925 to 1929. The most intensive work was performed during the summer of 1929.

HISTORY

Opius melleus was originally described in 1915 by Gahan (*2, p. 73*) from one female specimen found on Mount Washington, N. H., and was almost simultaneously described by Richmond (reported by Woods (*10*)) as *Biosteres rhagoletis* from 21 specimens reared from puparia of *Rhagoletis pomonella* from blueberries collected at Cherryfield, Me. In 1919 Gahan (*4*) cited *B. rhagoletis* as a synonym of *O. melleus*, and in the same year (*3*) he described two additional species, *O. richmondi* and *O. lectus*, both of which, he states, were swept from blueberry barrens in association with *O. melleus* and may also parasitize *R. pomonella*. Woods (*10*) was the first to rear *O. melleus* from puparia of the blueberry maggot, and he states that Severin reared it from the apple maggot. Good (*5*) described the method of oviposition into the apple maggot. Lathrop and Nickels (*7*) summarized the life history of *O. melleus*, gave tables indicating the percentage of parasitism of the blueberry maggot, and showed that this parasite could remain in the soil as long as four years and then successfully emerge as an adult.

During the present study *Opius melleus* proved to be the dominant parasite of the blueberry maggot in Washington County, Me., and was found in sufficient numbers to indicate that it is an important factor in the economy of its host. *O. richmondi*, reared in small numbers, was insignificant in comparison with *O. melleus*. No specimen of *O. lectus* was observed in the cages. If *O. lectus* parasitizes the blueberry maggot at all, it must be a minor species in eastern Maine.

SOME HOST RELATIONSHIPS

Rhagoletis pomonella infests blueberries (*Vaccinium* spp.) and huckleberries (*Gaylussacia* spp.), and also apples (*Malus* spp.) and haws (*Viburnum* spp.). The insect is commonly termed "blueberry

[1] Received for publication Apr. 21, 1932; issued February, 1933.
[2] Order Diptera, family Trypetidae.
[3] Reference is made by number (italic) to Literature Cited, p. 159.
[4] Order Hymenoptera, family Braconidae.
[5] The writers are indebted to A. B. Gahan for determinations of the species *Opius melleus* and *O. richmondi*.

maggot" or "apple maggot" according to the fruit with which it is associated. The two forms of the species appear to be ecologically distinct (*6*, *8*), but no morphological basis for separation has been discovered,[6] although the apple maggot is distinctly larger than the blueberry maggot in every stage.

Opius melleus parasitizes both the apple maggot and the blueberry maggot. It is a significant fact that specimens of the parasite from the apple maggot are distinctly larger than specimens reared from the blueberry maggot. (Fig. 1.) Other than size, no structural difference affords a basis for separation of the species from the different hosts. Apparently *O. melleus* exhibits a tendency toward the development of two divergent strains parallel to the ecological forms of the host.

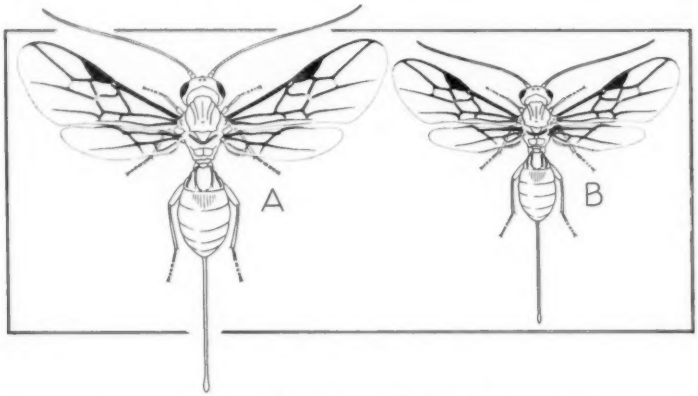


FIGURE 1.—*Opius melleus*; dorsal view of female with wings spread: A, From the apple maggot; B, from the blueberry maggot. × 8. Note the similarity of structure and the difference in size

LIFE HISTORY OF THE PARASITE IN RELATION TO THAT OF THE HOST

Opius melleus is well fitted to parasitize the blueberry maggot. The ovipositor of the female parasite is long enough to reach the host within the berry, and the life cycles of the two species coincide, to the advantage of the parasite. (Fig. 2.)

The flow of emergence of the adult parasites approximately parallels that of the flies, following it at an interval of 10 to 15 days. (Fig. 3.) Emergence of the females follows that of the males by 4 or 5 days. The earliest of the female parasites begin oviposition in 12 to 14 days after emergence, just as the maggots of the later instars appear in the berries in increasing numbers. The period of oviposition by the parasites coincides with the period of maximum abundance of maggots of the late second and third instars.

A portion of the host puparia remains in the soil for two years or more, thereby establishing a cycle of two years or longer. *Opius melleus* parallels this behavior, a portion of the population exhibiting a cycle of two or more years.

[6] Since this paper was written, C. H. Curran has described the blueberry maggot as a distinct species, *Rhagoletis mendax*, in American Museum Novitates No. 526, 1932. The validity of the species awaits confirmation by other dipterists.

The relationship existing between the blueberry maggot and its parasite, *Opius melleus*, is such as would be expected to occur between species in their native habitat, which has been occupied for an indefinitely long period under stable environmental conditions. Such conditions would permit the mutual adjustments which are exhibited by the maggot-parasite association.

LIFE HISTORY OF OPIUS MELLEUS

The investigation of the life history of *Opius melleus* was conducted largely as a series of careful field studies, but in certain phases a combination of field and laboratory methods was employed.

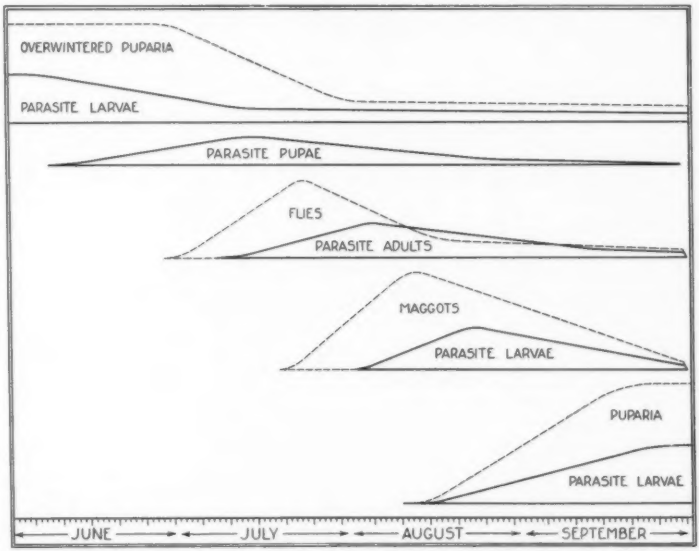


FIGURE 2.—Summary of life history of *Opius melleus* in relation to the blueberry maggot during an approximately normal season, Cherryfield, Me.

EMERGENCE OF ADULTS

Opius melleus spends the winter as a full-grown larva within the puparium of its host just beneath the surface layers of the soil. (Fig. 2.) Pupation takes place within the enveloping puparium about 30 days before the adult parasite emerges.

When *Opius melleus* emerges from the puparium, it is apparently fully pigmented and the wings are fully expanded and hardened. The insect works its way upward through the soil by movements of the head, body, and appendages. Parasites that were observed in a glass-front observation box had much more difficulty in emerging than did the flies. The parasites showed distinct signs of fatigue after climbing upward through 2 inches of dry sand, and no parasites were observed to emerge from a depth of 4 inches. The flies emerged from this depth with comparative ease.

The difficulty experienced by the emerging parasites suggested that parasitized maggots might pupate at a more shallow depth in the soil than do nonparasitized individuals. A study of 1,694 puparia indicated that there is no significant correlation between the percentage of parasitism and the depth at which the puparia are normally found.

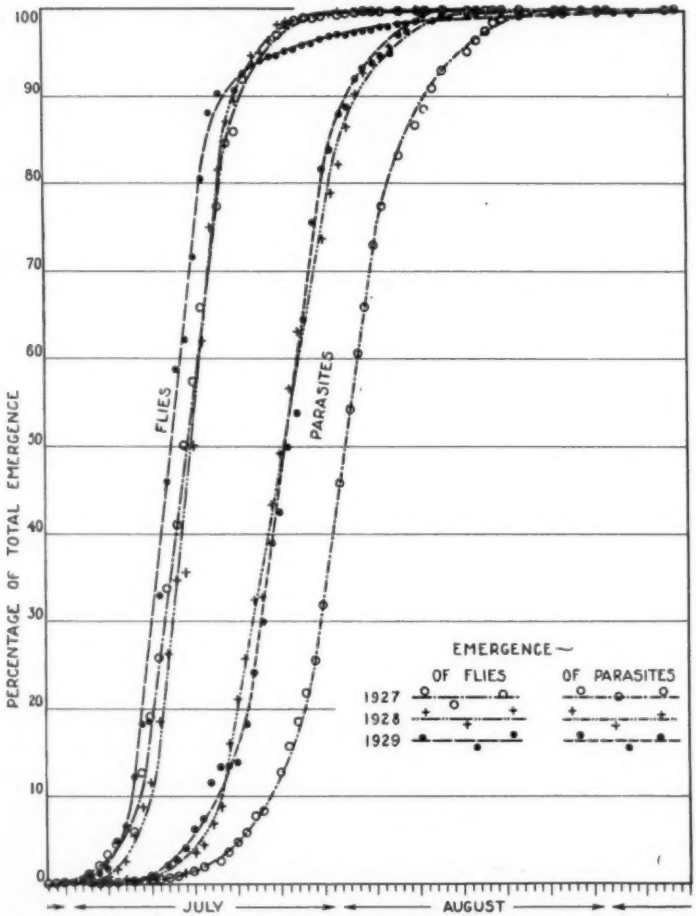


FIGURE 3.—Cumulative curves showing the emergence of adults of *Opius melleus* and its host during the seasons of 1927, 1928, and 1929

The fact that on an average 99 per cent or more of the puparia are found within 1½ inches of the surface of the soil makes it apparent that under field conditions the parasites have no serious difficulty in emerging from the soil.

The seasonal emergence of adults was studied by observations of the cages used in the studies of *Rhagoletis pomonella* by Lathrop and

Nickels (7). Blueberries heavily infested with maggots were placed on wire-screen trays over a series of plots during the late summer and autumn of each season. Each plot was 4 by 5 feet in size. The maggots dropped from the berries and entered the soil, where they pupated. During the following season each plot was covered by an emergence cage, and a careful record was made of the flies and parasites that emerged.

The emergence of flies and of parasites during the three seasons in which observations were made is best comprehended by a study of the accumulated emergence for each season. The records for the parasites are summarized in Table 1, and are illustrated graphically in Figure 3. It will be noted that the cumulative emergence curves have a characteristic form, depicting a slow rise early in the season, followed by a period of maximum emergence during which most of the adults appear, and a gradual cessation of emergence toward the end of the season.

TABLE 1.—*Summary of emergence records of adults of Opius melleus for three years, Cherryfield, Me.*

Year	Sex	Date of first emergence	Date of accumulated emergence in percentages indicated			Date of last emergence
			10	25	50	
1927	Male	July 3	July 21–22	July 27	July 30–Aug. 1	Sept. 17.
	Female	July 10	July 28–29	Aug. 1–2	Aug. 4–5	Sept. 14.
	Both		July 23–25	July 29	Aug. 1–2	
1928	Male	July 6	July 17–18	July 19–20	July 24–25	Aug. 24
	Female	July 15	July 20	July 23–24	July 28–29	Aug. 29.
	Both		July 18–19	July 21	July 25–26	
1929	Male	July 8	July 15–16	July 20–21	July 23–24	Aug. 11.
	Female	July 12	July 22–23	July 25–26	July 28–29	Aug. 12.
	Both		July 16–17	July 22–23	July 26	
			75	90	95	
1927	Male	July 3	Aug. 3	Aug. 8	Aug. 13	Sept. 17.
	Female	July 10	Aug. 8–10	Aug. 13–16	Aug. 18	Sept. 14.
	Both		Aug. 5–6	Aug. 12	Aug. 16	
1928	Male	July 6	July 28–30	Aug. 1–2	Aug. 4	Aug. 24.
	Female	July 15	Aug. 1–2	Aug. 6	Aug. 8–10	Aug. 29.
	Both		July 30–31	Aug. 3	Aug. 7	
1929	Male	July 8	July 27–28	July 30–31	Aug. 2–3	Aug. 11.
	Female	July 12	July 31–Aug. 1	Aug. 7–8	Aug. 9	Aug. 12.
	Both		July 29	Aug. 3	Aug. 7	

It is interesting to compare the emergence of the adult parasites with that of the flies. (Fig. 3.) Although the emergence of parasites is drawn out over a slightly longer period, the general form of the cumulative emergence curves is similar for both parasites and flies. The emergence curves of parasites during the seasons of 1928 and 1929 practically coincide, and might well be represented by a single line. The emergence of the parasites in 1927 occurred approximately seven days later than in 1928 and 1929, although the emergence of flies in 1927 coincided very well with that of 1928 and 1929. Probably the late emergence of parasites in 1927 was due to environmental influence of some kind, but the records of temperature and rainfall during the emergence periods do not afford any explanation.

PROPORTION OF THE SEXES

During the early part of the emergence period the adults of *Opius melleus* which appear in the cages are largely males. Later the percentage of females increases, and toward the end of the period the females outnumber the males. In the total emergence of adults during the season, the males predominate. During the three seasons for which records are available the females constituted an average of 37.68 per cent of the adults captured in the cages. (Table 2.) It would seem that this tendency to produce excessive numbers of males might reduce the effectiveness of the parasite. The production of excessive numbers of males has been observed in other opiine parasites (*9*), and is probably due in part to parthenogenetic reproduction. Unmated females of *O. melleus* were observed to attempt oviposition, and it is probable that parthenogenesis occurs commonly in the field.

TABLE 2.—*Proportions of the sexes of Opius melleus captured in emergence cages at Cherryfield, Me.*

Year of emergence	Males	Females	
	Number	*Number*	*Per cent*
1927	2,491	1,549	38.34
1928	367	195	34.70
1929	216	144	40.00
Average			37.68

CARRY-OVER IN THE SOIL FOR TWO OR MORE SEASONS

Like its host, the blueberry maggot, *Opius melleus* is capable of spending two or more seasons in the soil and then successfully emerging as an adult. From puparia formed in 1925 adults of *O. melleus* were observed to emerge during the summers of 1926, 1927, 1928, and 1929. Observations of emergence in eight cages over a period of three years are summarized in Table 3.

TABLE 3.—*Carry-over of Opius melleus in the soil to the second and third season (host puparia formed in 1926)*

Cage No.	1927		1928			1929			Total for the 3 years		
	Flies	Parasites	Flies	Parasites	Parasitism, as compared with 1927	Flies	Parasites	Parasitism, as compared with 1927	Flies	Parasites	Parasitism
	Number	*Number*	*Number*	*Number*	*Per cent*	*Number*	*Number*	*Per cent*	*Number*	*Number*	*Per cent*
A	3,937	462	79	30	6.49	2	0	0	4,018	492	10.91
B	4,676	65	67	11	16.92	2	1	1.54	4,745	77	1.60
2	2,564	42	46	8	19.05	2	0	0	2,612	50	1.88
3	1,401	82	76	40	48.78	9	6	7.32	1,486	128	7.93
5	475	20	35	7	35.00	5	0	0	515	27	4.98
8	2,691	687	273	146	21.25	23	12	1.75	2,987	845	22.05
9	2,365	997	358	320	32.10	59	14	1.40	2,782	1,331	32.36
11	2,498	566	532	191	33.75	22	8	1.41	3,052	765	20.04
Total	20,607	2,921	1,466	753	[a] 25.78	124	41	[a] 1.40	22,197	3,715	[a] 14.34

[a] Percentages based on totals.

OCCURRENCE OF ADULTS IN THE FIELD

The occurrence of adult parasites in the field was studied in the summer of 1929 by making counts on unit areas, using the method developed by Lathrop and Nickels (7) in the study of the blueberry maggot. The plot under observation was divided into 20 count areas, each 50 by 100 feet. During the period of abundance of the adult parasites five men familiar with the parasites made counts twice a week, usually on consecutive days. Each man, provided with a light frame which inclosed an area 25 inches square (approximately 0.0001 acre), selected a place in the first count area and set his frame in position. When everyone was ready, a signal was given, and each man studied his unit area intently to observe any adult parasites present. At the end of two minutes, when another signal was given, the study of the area was discontinued and each man recorded the number and, if possible, the sex of the parasites observed. The frames were then moved to new places in the first count area, where another count was made. This process was repeated in each of the 20 count areas, and a total of 200 counts covering a combined area of 0.02 acre was made. The total number of parasites observed in the 200 counts, multiplied by 50, therefore represented the number of parasites per acre present on the plot.

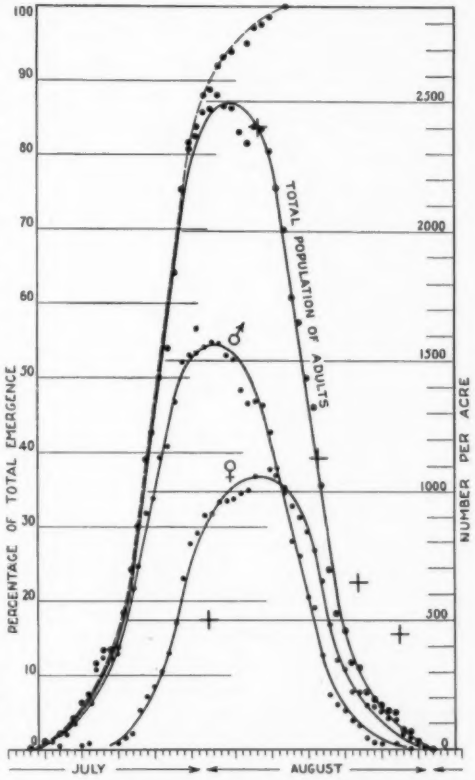


FIGURE 4.—Occurrence of adults of *Opius melleus* on experimental plot, Cherryfield, Me. Broken line indicates cumulative emergence of adults for the season of 1929. Upper curve shows the theoretical occurrence of adults throughout the season. The lower curves indicate the theoretical occurrence of adult males and females, respectively. The large crosses indicate the results of field counts of the total adult population on the dates indicated. All the population curves are based upon an average duration of life of 20 days. Note the agreement between the theoretical curves and the field counts

The results of the counts are presented in Table 4 and shown in Figure 4. With the method described by Lathrop and Nickels (7), the data from the field counts were plotted simultaneously with data from the emergence cages to construct Figure 4. A study of these curves brings out some interesting facts regarding the fluctuations of the

population of adult parasites. The numbers of parasites in the field rises rapidly as emergence proceeds. After the peak of occurrence is reached, the declining emergence and the increasing mortality result in a rapid decrease in the population. At the peak of occurrence there were present in the field more than 85 per cent of the total number of adults that emerged during the season. Theoretically, a total of approximately 2,800 or 2,900 *Opius melleus* adults per acre emerged on the area under observation. The males reached maximum numbers during the first few days in August, when approximately 1,500 individuals per acre were present. The maximum number of females occurred 7 to 10 days later with approximately 1,100 individuals per acre present in the field.

TABLE 4.—*Field counts of adults of Opius melleus, 1929*

Date	Adults of *Opius melleus*				Number per acre	
	Male	Female	Sex uncertain	Total	Each date	Average of pairs of counts
Aug. 1	8	3	0	11	550	500
Aug. 2	7	2	0	9	450	
Aug. 8	26	22	7	55	2,750	2,400
Aug. 9	21	16	4	41	2,050	
Aug. 16	15	12	0	27	1,350	1,125
Aug. 17	6	11	1	18	900	
Aug. 21	2	10	2	14	700	650
Aug. 23	5	7	0	12	600	
Aug. 27	4	5	1	10	500	450
Aug. 28	0	8	0	8	400	

LONGEVITY OF ADULTS

Two methods were used in an effort to determine the duration of life of adults of *Opius melleus:* (1) The commonly used laboratory method of confining individuals in glass vials, where they were supplied with dilute sugar solution on pieces of blotting paper; and (2) the method of the simultaneous study of field counts and emergence records (7).

The data presented in Table 5 indicate that the average length of life of adults in the laboratory was 17.5 days. In this study there was no significant difference between the average length of life of males and of females. The simultaneous study of data from the field counts and the emergence records, as shown in Figure 4, indicates an average length of life of approximately 20 days. The field counts showed an apparent increase in the average length of life of the adults toward the end of the season.

TABLE 5.—*Longevity of adults of Opius melleus in the laboratory, Cherryfield, Me., 1929*

	Number of adults living number of days indicated [a]																									Total
	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	
Males	1	0	0	1	2	2	3	3	5	6	7	6	11	6	8	20	17	18	22	7	5	3	2	6	1	0	1	163
Females	0	1	0	2	3	2	6	4	3	5	7	3	8	9	9	9	7	9	12	3	6	6	2	1	2	1	2	122
Total	1	1	0	3	5	4	9	7	8	11	14	9	19	15	17	29	24	27	34	10	11	9	4	7	3	1	3	285

[a] Average duration of life was 17.8 days for males, 17.1 days for females, and 17.5 days for both.

OVIPOSITION

Females of *Opius melleus* confined in vials in the laboratory attempted oviposition within as short a time as five days after emergence. However, this does not necessarily mean that oviposition would take place so soon in the field. Data obtained from the emergence cages and from careful field observations led to the conclusion that the average preoviposition period in the field is considerably longer than the laboratory studies indicate.

During the season of 1929 adult females began emerging in numbers about July 20, as shown in Figure 4. Parasitism of the maggots did not develop in the field until about August 2, as shown in Figure 5 and Table 8. This apparently indicates that there is a preoviposition period of approximately 13 days. It may be argued that, since the eggs are deposited in maggots of the late second instar and the third instar, the parasites began oviposition as soon as host material was available, and the delay was not due to a definite preoviposition period. However, the definite emergence of the female parasites 10 to 13 days prior to the development of suitable host larvae makes it appear probable that in the field there is a normal preoviposition period of approximately this duration.

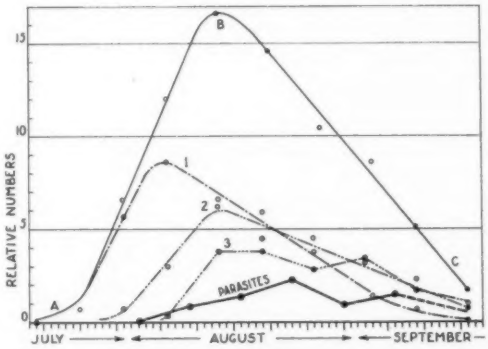


FIGURE 5.—Fluctuations in the population of immature stages of *Opius melleus* in relation to the population of blueberry maggots on experimental plot, 1929. Curve A–B–C indicates the total maggot population in terms of units per 100 berries. Curves 1, 2, and 3 show the population of maggots in the first, second, and third instars, respectively. The heavy line indicates the relative number of *O. melleus* (immature stages). The data concerning the populations of the host are from Lathrop and Nickels (7)

The process of oviposition by the parasite was observed a few times in the field and rather commonly in the laboratory. The female examines the berry carefully with her antennae. When she locates the maggot, she raises her body and brings the ovipositor down into position. (Fig. 6, A–D.) Then, by lowering the body, she thrusts the setae of the ovipositor deep into the berry. After a momentary pause, she removes the ovipositor from the berry and resumes her normal activity. The entire process of oviposition occupies 10 to 15 seconds.

In her search for host larvae in which to oviposit, the female parasite is apparently guided by the vibration produced by the movements of the maggots within the berries. The parasite could be induced to attempt oviposition into the empty skin of a blueberry by making movements beneath the blueberry skin with a needle or a pair of forceps to simulate the movements of a maggot. Maggots placed beneath the skin of a blueberry in a Petri dish stimulated oviposition, but the parasites made no attempt to oviposit directly into naked maggots crawling in the dish.

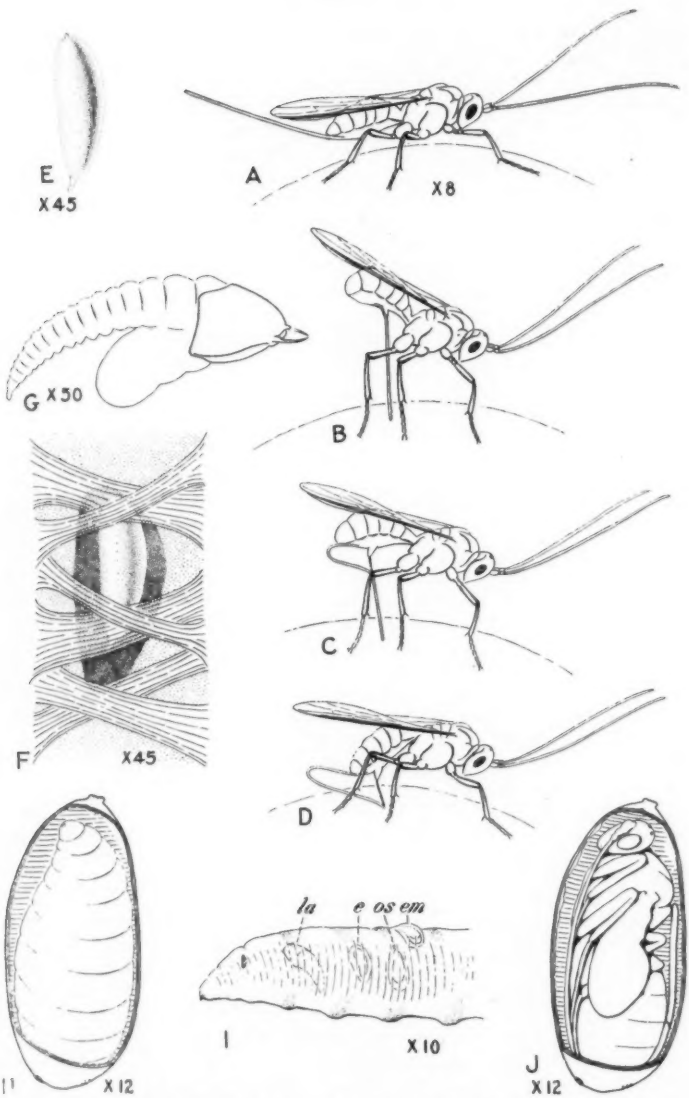


FIGURE 6.—A, *Opius melleus* on the surface of a blueberry, searching for a maggot in which to oviposit; B, C, D, three stages in the act of oviposition; E, egg of *O. melleus* removed from a blueberry maggot; F, egg of *O. melleus* viewed from the inner surface of the body wall of the blueberry maggot, showing the position of the egg between the epidermis and the muscle fibers; the egg is surrounded by a pellucid area or "blister," which shows dark in the illustration; G, newly hatched larva of *O. melleus*, removed from the body of a blueberry maggot; H, mature larva within the puparium, from which a portion of the wall has been removed; I, anterior portion of a blueberry maggot, showing a recently deposited egg (*e*), an egg containing a well-developed embryo (*em*), a newly hatched larva (*la*), and an ovipositor scar (*os*) of the parasite, *O. melleus;* J, pupa of *O. melleus* within the puparium of the blueberry maggot

The egg may be deposited within the maggot in a dorsal, ventral, or lateral position and anywhere from the anterior to the posterior extremities. Usually the egg is found just beneath the skin of the maggot, between the muscle fibers and the outer cuticle. (Fig. 6, E, F.) It is possible that the parasite eggs may be placed in other parts of the maggot also, where they are more difficult to see, and therefore where they are less frequently discovered. One egg was observed loose in the body cavity of a maggot, and was dissected out. This egg may have been placed just beneath the skin of the maggot, and accidentally dislodged and set free in the body cavity.

The newly deposited egg is inconspicuous, but as the embryo develops the egg increases in size, and usually produces a somewhat more readily observed "blister" on the skin of the maggot. (Fig. 6, I.) The point of entrance of the ovipositor is frequently marked by a darkened point on the integument of the maggot. The darkened spot could not always be discovered, however, and it could not be determined that the egg was held in place by any cementing material. Apparently the maggots were frequently pierced by the parasite without an egg being deposited. At least, what appeared to be oviposition scars were commonly observed where no egg could be discovered. That the ovipositor may sometimes be thrust entirely through the maggot is indicated by the fact that oviposition scars are sometimes found in pairs on opposite sides or nearly opposite sides of the maggot.

Oviposition scars in the third-instar maggots may persist in the puparia, and apparently the scars sometimes provide points of entry of bacterial or fungous organisms, which attack the tissues within.

It is not uncommon to find two or more eggs in one maggot. A maggot was once observed to contain four first-instar parasite larvae. Apparently when more than one larva is present in a puparium there is a struggle for survival. In one instance a puparium was dissected and found to contain two battling parasite larvae. Never has more than one mature larva been observed in a puparium.

INCUBATION PERIOD

The incubation period of the egg of *Opius melleus* was not satisfactorily determined in this study. Willard (*9*) found that the eggs of *O. fletcheri* in Hawaii hatched in 37 to 40 hours after oviposition. As might be expected, the egg of *O. melleus* in Maine required a much longer time. Limited observations indicate that the egg of *O. melleus* requires an incubation period of from three to six days. Some eggs deposited in the nearly mature maggots were observed to hatch within the puparia.

LARVAL DEVELOPMENT

The first-instar larva (fig. 6, G) is actively motile, and swims freely within the body cavity of the host. In this stage the larva is provided with a pair of long, sharply pointed jaws. The parasite larva has never been observed to transform to the second instar until after the formation of the host puparium. Then the larval development of the parasite usually proceeds rapidly. In most cases under observation the parasite larvae attained the fourth instar within 10 days after the puparium was formed. Larval development may be delayed,

however, one puparium being observed to contain two living parasite larvae in the first instar at least 4 weeks after the formation of the puparium. The larval transformations are complete, nevertheless, before freezing temperature occurs.

The parasite larva in the second, third, and fourth instars is inactive, and is without conspicuous appendages. The host tissues appear to undergo normal histolysis after the formation of the puparium. The pupal structures are not formed, however; instead, the substance of the host is ingested by the growing parasite larva. The mature parasite larva almost completely fills the puparium. (Fig. 6, H.) Usually the walls of the puparium, when moist, are translucent, and the presence of a parasite larva may be detected by external examination of the puparium. The exuviae of the preceding instars are pressed against the wall of the puparium by the larva, and the old head capsule of the first instar may often be seen through the wall of the puparium containing a mature larva of *Opius melleus*. It is not uncommon to find two or more head capsules of first-instar larvae within a single puparium (in a few cases four head capsules were found), an indication of the competition of several larvae and the survival of but one.

THE IMPORTANCE OF OPIUS MELLEUS

The quantitative study of the relation of *Opius melleus* to its host is as difficult as it is interesting. The problem consists of two phases, the determination of the percentage of parasitism and the evaluation of the effect of the parasite upon the economy of its host.

THE DETERMINATION OF PERCENTAGE OF PARASITISM

In estimating the degree of parasitism the objective is to determine the percentage of blueberry maggots attacked by *Opius melleus* throughout the season, thereby indicating the proportion of the total population of maggots destroyed by the parasite. In view of the life habits of the parasite, and the single-generation cycle of both parasite and host, the ideal method of estimating the percentage of parasitism would be the examination of a representative sample of puparia taken from the soil of the area being studied. The sample of puparia should be obtained during the dormant period when both parasite and host are in a hibernating condition. Under normal conditions in the field, however, large quantities of soil must be examined to obtain a very few puparia of the blueberry maggot, even on areas supporting a large maggot population. It was therefore found impracticable to obtain a representative sample of puparia, and it was necessary to resort to other methods for making quantitative estimates of parasitism.

Two methods were used to determine the empirical percentage of parasitism of maggots from samples of blueberries: (1) Placing the berries over pupation plots, and determining the ratio of parasites to flies captured in emergence cages placed over the plots during the following season; and (2) examining puparia obtained from samples of blueberries. The results of two series of determinations are summarized in Tables 6 and 7.

TABLE 6.—*Parasitism of the blueberry maggot by Opius melleus*

[Records from emergence cages, 1927]

Cage No.	Flies	Parasites	Parasitism	Cage No.	Flies	Parasites	Parasitism
	Number	*Number*	*Per cent*		*Number*	*Number*	*Per cent*
A	3,937	462	10.50	8	2,691	687	20.34
B	4,676	65	1.37	9	2,365	997	29.66
1	1,192	17	1.41	10	1,101	302	21.53
2	2,564	42	1.61	11	2,498	566	18.47
3	1,401	82	5.53	12	2,447	142	5.49
4	1,085	90	7.66	13	1,567	297	15.93
5	475	20	4.04	14	405	64	13.65
6	1,738	169	8.86				
7	711	38	5.07	Total	30,853	4,040	[a]11.58

[a] Percentage based on totals.

TABLE 7.—*Parasitism of the blueberry maggot by Opius melleus*

[Determinations made by examination of puparia]

Lot No.	Date berries were picked	Puparia examined	Puparia parasitized		Source of berries
	1927	*Number*	*Number*	*Per cent*	
1	Aug. 16	340	133	39.12	A homogeneous sample picked on poorly tended land. A high percentage of the berries was infested with maggots.
2	do	224	85	37.95	
3	Aug. 20-24	118	24	20.34	Accumulated as left-overs of samples from check plots.
4	do	77	19	24.68	Accumulated as left-overs from samples from plots dusted with calcium arsenate.
5	Aug. 25	188	39	20.75	Picked on brush-free portions of check plot.
6	do	195	56	28.72	Picked on bushy portion of check plot.
7	do	61	2	3.28	Picked on brush-free portions of plot dusted with calcium arsenate.
8	do	115	23	20.00	Picked on bushy portions of check plot.
9	Aug. 26	196	93	47.45	A homogeneous sample picked on poorly tended land. A high percentage of the berries was infested with maggots.
10	do	257	127	49.42	

At first thought it would seem that a determination of the percentage of parasitism of a series of samples, such as that shown in Tables 6 and 7, would give a reasonably satisfactory basis for estimating the efficiency of *Opius melleus*, especially if the samples are large enough to yield reliable data, are numerous enough to indicate the range of variation, and extend fairly well over the season. A more thorough consideration, however, reveals several reasons why such a series in itself can not indicate, even approximately, the true numerical relation of *O. melleus* to its host.

Each of the determinations of parasitism shown in Tables 6 and 7 indicates the percentage of the entire population of maggots of all stages that were parasitized at the time the sample of berries was picked. As only the maggots of the late second and third instars are subject to parasitism, however, it is evident that a determination of the percentage of parasitism of the entire population of maggots does not give a fair indication of the effective parasitism. Willard (*9*), in working with *Opius fletcheri* as a parasite of the melon fly in Hawaii, observed a similar situation, and overcame the difficulty by basing estimates of parasitism only upon maggots which emerged from the cucumbers—within which the hosts fed—during the first two to four days after collection. Such a method can not be em-

ployed in the case of *O. melleus*, because the blueberry maggot remains in the berries for an abnormally long time if the berries are picked and placed where they are not subject to normal weathering. Even were it possible to base the estimate on the percentage of parasitism of susceptible maggots only, a series of samples picked from various localities and at different dates would give little indication of the efficacy of *O. melleus*. The reason for this is that each sample (Tables 6, 7, and 8) represents a cross section of the maggot-parasite association considered as a static population, and shows the percentage of parasitism existing in this supposedly static population at the moment that the sample was taken. In reality the maggot-parasite population is by no means in a static condition. The condition might more properly be regarded as a moving stream terminating in a reservoir, or rather as a combination of two streams, one of maggots and the other of parasites, each flowing at a different rate. With such a conception in mind, it is clear that a cross section of such a composite stream, or even a series of cross sections, will not indicate the relative percentage of maggots and parasites in the reservoir in which the stream terminates unless the relative rates of flow of maggots and of parasites are considered.

TABLE 8.—*Seasonal study of parasitism of the blueberry maggot by Opius melleus on experimental plot, Cherryfield, Me., 1929*

Date	Parasitism of puparia from field samples	Total maggot population per 100 berries [a]	Relative numbers of parasites present throughout season	Maggots present per 100 berries [b]	Maggots parasitized
	Per cent	*Number*		*Number*	*Per cent*
Aug. 2	0.50	10.8	0.05	8.2	0.61
Aug. 9	5.50	16.2	.89	14.5	6.14
Aug. 16	8.36	16.8	1.41	16.2	8.70
Aug. 23	16.98	13.5	2.29	13.2	17.35
Aug. 30	9.45	10.1	.96	9.8	9.80
Sept. 6	22.91	6.6	1.51	6.5	23.23

[a] Including eggs and maggots. [b] Not including eggs.

Clausen and King (*1, p. 9–10*) were confronted by a somewhat parallel problem in their work with *Centeter cinerea* Ald., a parasite of *Popillia japonica* Newm. These authors showed that the host was killed within six days after the parasite egg was deposited; therefore, practically all beetles bearing eggs on any given date are dead within six days from that time, and the parasitized beetles in the field at a later date represent an additional percentage of the total.

> To illustrate * * *. On July 14, 35 per cent bore eggs, these being replaced on the 20th by 45 per cent of the remainder, the latter in turn being replaced by 48.5 per cent of those remaining on the 26th. Thus, theoretically, the parasitism effected was 35 plus 29 plus 17 per cent, successively, during the period of abundance, totaling approximately 81 per cent of the entire infestation.

In the case of *Opius melleus*, the total parasitism can not be deduced by a summation of this kind. *O. melleus* does not immediately destroy its host, and the length of time that the parasite spends in the host larva is variable.

An interesting view of the problem is presented by the data in Table 8, shown graphically in Figure 5. A large sample of blueberries was collected from the experimental plot once a week throughout the season. The berries were placed over sand boxes. In the autumn, after pupation was complete, the puparia were collected and examined to determine the percentage of parasitism in each of the weekly samples. In considering these data it should be borne in mind that the parasite is present during only a portion of the life of the maggot. Therefore, the effective parasitism is somewhat higher than the ratio of parasites to total maggots in the population. If the relative duration of life of the maggot and of the parasite egg and larva combined were known, the total parasitism for the season might be determined from the data summarized in Figure 5. For example, if the parasite eggs were laid only in the newly hatched maggots, then the parasite would be present throughout the life of the maggot, and the curve showing the relative number of parasites must coincide throughout the season with the curve (A–B–C) of the total maggot population to indicate 100 per cent parasitism. If the duration of life of the parasite within the maggot were equal to that of the third instar of the maggot, then the parasite curve in Figure 5 should coincide with curve 3 throughout the season to indicate 100 per cent parasitism. Actually, the parasite eggs are deposited at any time after the maggot approaches the third instar. Many eggs are deposited in maggots of the second instar; others are deposited in full-grown maggots on the point of leaving the berries. It seems probable that the average duration of life of the parasite within the maggot is nearly equal to the duration of the third instar of the host. It would seem, therefore, that a fairly accurate estimate of the total seasonal parasitism of the blueberry maggot by *Opius melleus* on the plot under observation might be arrived at by a determination of the relative areas inclosed by curve 3 and by the "parasite" curve in Figure 5. A comparison of the areas of the two figures by means of a planimeter indicated a ratio of 1 to 0.4919. There was, therefore, a total seasonal parasitism of 49.19 per cent on the experimental area under observation.

EFFECT OF OPIUS MELLEUS UPON THE ECONOMY OF ITS HOST

If the determination of the total parasitism by *Opius melleus* is difficult, the estimation of the effect of the parasite upon its host is much more so, and in the present state of knowledge must be little more than careful conjecture.

Discussing the importance of *Opius fletcheri*, Willard (*9, p. 434*) states: "While this parasite alone will never exercise a complete control over the melon fly in Hawaii, it has already proved of much value by decreasing the numbers of this pest considerably." Data given by Willard show that the highest parasitism obtained in his study was 29 per cent of the susceptible larvae (melon-fly larvae emerging from cucumbers within two to four days after picking). The average parasitism was 18.1 per cent.

The data presented in the present study show parasitism by *Opius melleus* as high as 49 per cent. The average parasitism in Table 6 is 11.58 per cent and in Table 7 is 29.17 per cent. These estimates are based on the entire maggot population of the berries at the time the

samples were picked, and the total parasitism for the season has been shown to be considerably higher than estimates based upon the total maggot population of isolated samples.

It is evident that the percentage of parasitism of the blueberry maggot by *Opius melleus* is as great as that which occurs in the case of some parasites which are considered important factors in reducing the population of imported insect pests. The percentage of parasitism alone, however, does not necessarily indicate the potential value of the parasite, for the effect of the parasite upon the abundance of the host is dependent upon a number of other factors, such as comparative rates of reproduction and ecological competition.

Opius melleus and its host, the blueberry maggot, are both native to eastern Maine. The association between the two species has no doubt existed sufficiently long for the complete establishment of mutual adjustments between the economy of the parasite and that of its host. It is not to be supposed, therefore, that *O. melleus* is actively reducing the population of the blueberry maggot, or that it will ever exert such an effect unless there should occur some marked change in the environment of the species. It seems clearly indicated, however, that this parasite is an important ecological factor in the balance which apparently exists in the blueberry-maggot association.

SUMMARY

In connection with an investigation of the blueberry maggot in eastern Maine during the years 1925 to 1929, a study was made of the biology of the parasite *Opius melleus* Gahan.

Opius melleus is well fitted to parasitize the blueberry maggot The ovipositor of the female parasite is long enough to reach the host within the berry, and the life cycles of the two species parallel each other, to the advantage of the parasite.

The full-grown larva of the parasite spends the winter within the puparium of the host just beneath the surface layers of the soil Pupation takes place within the puparium about 30 days before the adult parasite emerges. Adults begin to emerge during the first two weeks in July, and in normal seasons emergence is almost complete by the middle of August. Oviposition begins early in August and continues during the period of abundance of suitable host larvae.

During the three seasons for which records are available the females constituted an average of 37.68 per cent of the adults captured in emergence cages.

Like its host, the blueberry maggot, *Opius melleus* is capable of remaining in the soil for two or more seasons and then successfully emerging as an adult.

Fluctuations of the population of adult parasites were studied by counts of unit areas in the field. The males reached maximum numbers during the first few days in August, when approximately 1,500 individuals per acre were present. The maximum number of females occurred 7 to 10 days later, with approximately 1,100 individuals per acre.

Laboratory studies indicated that the average length of life of the adult parasites was 17.5 days, with no significant difference in the longevity of males and females. The simultaneous study of data

from field counts and the emergence records indicates an average length of life of approximately 20 days.

The female parasite deposits the egg directly into the body of the maggot. The egg hatches within three to six days. The parasite larva swims freely in the body cavity of the maggot, without injury to the host, until the host puparium is formed. Parasite larvae may be found in the maggots from early August until frost occurs—usually in late September or early October.

After the formation of the puparium by the host, the parasite larva undergoes rapid development, and the larval transformations are complete before freezing temperatures occur.

Records of adults captured in emergence cages indicated parasitism of the blueberry maggot by *Opius melleus* ranging from 1.37 to 29.66 per cent. Examinations of puparia indicated parasitism ranging from 3.28 to 49.42 per cent. A thorough study of the problem shows that the determination of the percentage of parasitism of isolated samples does not indicate the effective seasonal parasitism by *O. melleus*. The most accurate estimate of the total seasonal parasitism was made by plotting the seasonal populations of maggots of the third instar and of parasites, and comparing the areas inclosed by the respective curves. (Fig. 5.)

The percentage of parasitism alone, however, does not necessarily indicate the potential value of the parasite in reducing the population of the blueberry maggot, for a number of other factors, such as relative rates of reproduction and ecological competition, must be taken into consideration. Inasmuch as both the parasite and its host are native to eastern Maine, their association has no doubt existed sufficiently long for the establishment of mutual adjustments in their economy. *Opius melleus* is not actively reducing the population of the blueberry maggot, and it will probably never have this effect unless some marked change in the environment of the species should occur. It seems clearly indicated, however, that this parasite is an important ecological factor in the balance which apparently exists in the blueberry-maggot association.

LITERATURE CITED

(1) Clausen, C. P., and King, J. L.
1927. The parasites of popillia japonica in japan and chosen (korea) and their introduction into the united states. U. S. Dept. Agr. Bul. 1429, 56 p., illus.

(2) Gahan, A. B.
1916. A revision of the north american ichneumon-flies of the subfamily opiinae. U. S. Natl. Mus. Proc. 49: 63–95, illus.

(3) ———
1919. Descriptions of seven new species of opius hymenoptera–braconidae. Ent. Soc. Wash. Proc. 21: 161–170.

(4) ———
1920. New reared parasitic hymenoptera with some notes on synonymy. U. S. Natl. Mus. Proc. 55: 113–128, illus.

(5) Good, C. A.
1916. A few observations on the apple maggot parasite—biosteres rhagoletis, richmond. Canad. Ent. 48: 168.

(6) Lathrop, F. H., and Nickels, C. B.
1931. The blueberry maggot from an ecological viewpoint. Ann. Ent. Soc. Amer. 24: 260–281, illus.

158010—33——5

(7) LATHROP, F. H., and NICKELS, C. B.
1932. THE BIOLOGY AND CONTROL OF THE BLUEBERRY MAGGOT IN WASHINGTON COUNTY, MAINE. U. S. Dept. Agr. Tech. Bul. 275, 77 p., illus.

(8) PATCH, E. M., and WOODS, W. C.
1922. THE BLUEBERRY MAGGOT IN WASHINGTON COUNTY. Maine Agr. Expt. Sta. Bul. 308, p. [77]–92, illus.

(9) WILLARD, H. F.
1920. OPIUS FLETCHERI AS A PARASITE OF THE MELON FLY IN HAWAII. Jour. Agr. Research 20: 423–438, illus.

(10) WOODS, W. C.
1915. BIOSTERES RHAGOLETIS RICHMOND, SP. N., A PARASITE OF RHAGOLETIS POMONELLA WALSH. Canad. Ent. 47: 293–295, illus.

BASE-EXCHANGE MODIFICATIONS OF A LEONARDTOWN SILT LOAM UNDER FERTILIZER AND CROP CONTROL[1]

By R. S. HOLMES

Associate Chemist, Division of Soil Chemistry and Physics, Soil Investigations, Bureau of Chemistry and Soils, United States Department of Agriculture

INTRODUCTION

The extensive examination of the colloid of Leonardtown silt loam, the dominant type of the Leonardtown soil series, by Holmes [2] revealed the fact that the colloid of this soil is remarkably constant in composition so far as the main analytical constituents are concerned, but that the basic and acidic components are rather variable. The Maryland Agricultural Experiment Station has for the last 12 years been conducting fertilizer treatment tests on a series of plots of Leonardtown silt loam situated about three-quarters of a mile east of La Plata, Charles County, Md. This area is extremely uniform in topography,

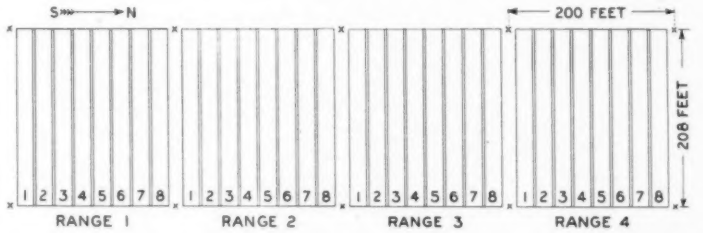


FIGURE 1.—Ranges and plots at the Maryland Agricultural Experiment Station, soil-fertility investigations, La Plata field. Only plots 1, 2, and 4 in each range were studied. The alleys between the ranges are 18 feet wide; the plots, 21 feet wide with 2-foot alleys between them. X indicates iron stakes set in concrete

and when the experiments were begun was very unproductive. The treatments given certain plots resulted in a marked alteration of crop yields. This area, therefore, seemed to offer special opportunity for a study of the base-exchange relationships between the untreated soil and the soil with improved fertility.[3] It was hoped that such a study might reveal the changes in chemical composition of the colloid which were responsible for the marked alteration of crop yields effected by the soil treatment.

DESCRIPTION OF AREA AND SOIL TREATMENT

The soil under examination is a very level and apparently uniform area. It was divided into ranges and plots as indicated in Figure 1. In each range the examination was limited to the most productive

[1] Received for publication Apr. 25, 1932; issued February, 1933.

[2] HOLMES, R. S. VARIATIONS OF THE COLLOIDAL MATERIAL IN TYPICAL AREAS OF THE LEONARDTOWN SILT LOAM SOIL. Jour. Agr. Research 36: 459–470. 1928.

[3] The data concerning the plots were placed at the writer's disposal through the kindness of Prof. J. E. Metzger and E. H. Schmidt, of the agronomy department of the University of Maryland.

plots numbered 1 and 2, and to the check plots numbered 4. In October, 1920, preliminary to starting the work, all the plots received a presumably uniform addition of limestone at the rate of 2 tons an acre. The operation of the plots began in the autumn of 1920. The whole series of plots was cultivated under the same 4-year rotation—corn, soybeans, wheat, and grass. The plots, when in corn, were given a cover-crop treatment of rye, which furnished green manure for the soybeans. Plot 1 in each range received, previous to corn planting, 5 tons of manure an acre, together with 1,000 pounds of ground rock phosphate; each wheat crop received 5 tons of manure only. Plot 2 in each range received 10 tons of manure an acre for corn, and 400 pounds of superphosphate for each wheat crop. At the time of sampling, October, 1930, the total manure that had been applied to plot 1 in each range was 26.25 tons an acre; and to plot 2, 25 tons an acre. Plots 1 had received 2,500 pounds of rock phosphate and plots 2, 1,200 pounds of superphosphate an acre. The effect of this treatment on the crop yield is indicated in Table 1 by the average yields for the 4-year period 1926–1929 and the yields for 1931.

TABLE 1.—*Yields on La Plata plots*

Year	Plot No.	Wheat	Corn	Soybeans	Hay	Year	Plot No.	Wheat	Corn	Soybeans	Hay
		Bush.	*Bush.*	*Bush.*	*Tons*			*Bush.*	*Bush.*	*Tons* [a]	*Tons*
4-year period 1926–1929	1	17.51	36.61	14.03	2.28	1931	1	30.2	59.1	1.41	1.00
	2	14.15	36.72	14.19	1.34		2	26.7	47.7	1.18	.65
	4	8.16	15.22	5.75	.83		4	11.0	27.9	.80	.30

[a] The soybean crop for 1931 was harvested as hay.

Although the growing season of 1931 was especially favorable for certain crops, and the actual yields of wheat and corn greatly exceeded the 4-year average, the difference between the plots which had been fertilized and the check plots is equally well marked.

COLLECTION AND EXAMINATION OF SAMPLES

By means of a King sampling tube 20 samples were taken along the longitudinal center of each plot to the mean plow depth, about 5 inches. These samples were thoroughly mixed, and the composite material examined. Range 2 was in corn, and some difficulty was experienced in obtaining subsamples of uniform depth, because of surface irregularities. Range 3 was in soybeans, and the surface was in a very pulverulent condition, which also rendered accurate sampling difficult. Range 1, in clover, and range 4, in wheat, were more level and compact, and the samples from these ranges doubtless represent the mean soil values better than those from ranges 2 and 3. The samples were subjected to mechanical analysis by the pipette method.[4] The data so obtained are given in Table 2.

[4] OLMSTEAD, L. B., ALEXANDER, L. T., and MIDDLETON, H. E. A PIPETTE METHOD OF MECHANICAL ANALYSIS OF SOILS BASED ON IMPROVED DISPERSION PROCEDURE. U. S. Dept. Agr. Tech. Bul. 170, 23 p., illus. 1930.

TABLE 2.—*Mechanical analyses of composites from different soil plots*

Range No.	Plot No.	Fine gravel, 2–1 mm	Coarse sand, 1–0.5 mm	Medium sand, 0.5–0.25 mm	Fine sand, 0.25–0.1 mm	Very fine sand, 0.1–0.05 mm	Silt, 0.05–0.005 mm	Clay, 0.005–0 mm	Inorganic colloid, 0.002–0 mm	Loss by H_2O_2 treatment
		Per cent	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*
1	1	1.1	4.5	7.1	9.6	5.4	51.4	20.9	12.0	1.8
	2	.8	4.4	6.9	8.8	5.6	52.6	20.9	11.9	2.6
	4	.6	4.5	7.1	8.8	5.2	54.4	19.4	11.1	1.5
2	1	.7	4.3	8.0	10.5	6.0	52.8	17.7	10.6	1.8
	2	.8	4.2	8.5	11.0	5.6	50.8	19.1	11.7	1.9
	4	.4	3.7	8.4	11.3	5.9	49.1	20.6	13.2	1.6
3	1	1.0	3.9	7.7	10.5	6.0	51.0	19.9	12.5	1.8
	2	.7	4.6	8.3	11.3	6.4	50.1	18.7	11.6	1.4
	4	.7	4.8	8.3	11.3	6.8	50.0	18.1	11.3	1.2
4	1	.5	3.9	7.5	10.0	6.5	52.6	19.1	11.2	2.0
	2	1.1	3.9	6.9	9.6	6.1	51.6	20.8	12.3	1.8
	4	.6	5.0	6.7	9.1	6.5	53.0	18.1	10.8	1.4

The samples were not subjected to complete chemical analysis, nor were the colloids analyzed because of the known character of the material.[5]

Two hundred grams of each sample was digested with 2 liters of 0.05 normal hydrochloric acid for 18 hours and then filtered and washed by means of a Pasteur-Chamberland filter. The filtrate was evaporated and analyzed. This procedure may be expected, as shown by Mattson,[6] to extract essentially the same base-exchange and acid-exchange materials as would be obtained by electrodialysis. The results obtained, expressed as milliequivalents per 100 g[7] of dry soil, are given in Table 3.

TABLE 3.—*Constituents of soil soluble in N/20 hydrochloric acid*

[Results expressed in milliequivalents per 100 g of air-dry soil]

Range No.	Plot No.	Ca	Mg	K	Na	Mn	Al	Fe	Si	P_2O_5
1	1	3.36	0.30	0.17	0.09	0.68	6.66	0.34	0.50	0.22
	2	2.28	.27	.13	.06	.82	4.65	.35	.53	.05
	4	2.22	.21	.07	.07	.56	4.26	.30	.60	.02
2	1	4.98	.47	.26	.27	.51	13.30	.75	1.42	.20
	2	5.78	.63	.22	.28	.59	11.30	.64	1.92	.06
	4	2.67	.33	.14	.38	.51	11.60	.45	1.52	.02
3	1	5.15	.63	.23	.23	.73	16.30	.53	2.12	.21
	2	3.06	.58	.22	.24	.56	11.88	.41	1.82	.06
	4	6.32	.80	.15	.24	.39	9.65	.37	1.76	.02
4	1	4.54	.47	.20	.08	.93	6.88	.37	.80	.28
	2	2.73	.35	.15	.07	.65	5.29	.27	.60	.06
	4	2.00	.29	.07	.08	.45	4.18	.22	.60	.03
	Average of plots numbered 1	4.51	.47	.22	.17	.71	10.78	.50	1.21	.23
	Average of plots numbered 2	3.46	.46	.18	.16	.65	8.28	.42	1.22	.06
	Average of plots numbered 4	3.30	.41	.11	.19	.48	7.42	.33	1.12	.02

The base-exchange components were also determined by leaching 100 g of each of the samples with 1 liter of normal ammonium acetate solution of pH 7. The procedure followed was that of Schollenberger

[5] HOLMES, R. S. Op. cit.

[6] MATTSON, S. ELECTRODIALYSIS OF THE COLLOIDAL SOIL MATERIAL AND THE EXCHANGEABLE BASES. Jour. Agr. Research 33: 553–567, illus. 1926.

[7] g is the abbreviation for gram or grams recently adopted by the Style Manual for U. S. Government printing.

and Dreibelbis.[8] The leachate was evaporated to dryness and analyzed. The results obtained are given in Table 4.

TABLE 4.—*Constituents of soil soluble in normal ammonium acetate solution*

[Results expressed in milliequivalents per 100 g of air-dry soil]

Range No.	Plot No.	Ca	Mg	K	Na	Mn	Al	Si
1	1	2.87	0.53	0.16	0.19	0.07	0.52	0.10
	2	2.95	.45	.15	.19	.06	.48	.13
	4	3.08	.43	.11	.15	.06	.42	.04
2	1	4.00	.54	.20	.40	.19	.60	.17
	2	5.35	.72	.12	.38	.17	.21	.43
	4	2.86	.37	.10	.46	.17	.62	.24
3	1	4.10	.50	.14	.43	.06	.56	.25
	2	3.50	.48	.16	.43	.14	.60	.32
	4	6.83	.47	.06	.38	.06	.38	.30
4	1	6.28	.58	.21	.16	.07	.50	.09
	2	2.97	.62	.15	.17	.07	.41	.16
	4	2.21	.35	.10	.18	.07	.50	.36
	Average of plots numbered 1	4.31	.54	.18	.29	.10	.54	.15
	Average of plots numbered 2	3.69	.57	.14	.29	.11	.42	.26
	Average of plots numbered 4	3.74	.40	.09	.29	.09	.48	.23

In addition to these examinations for base-exchange materials, the soils were examined to determine a number of other properties in respect to which the fertilizer and crop treatment might be expected to produce differences from the check plots. These determinations and the resulting values are given in Table 5.

TABLE 5.—*Miscellaneous chemical and physical determinations on Leonardtown silt loam*

Range No.	Plot No.	Organic matter	N	NO_3	Organic matter / Nitrogen	Carbon / Nitrogen	P_2O_5	Soluble P_2O_5 / Total P_2O_5
		Per cent	*Per cent*	*Per cent*			*Per cent*	*Per cent*
1	1	2.08	0.087	0.0019	23.9	13.6	0.083	6.15
	2	1.75	.079	.0012	22.2	13.0	.058	2.08
	4	1.41	.060	.002	22.1	13.5	.050	1.00
2	1	1.91	.087	.0016	22.0	11.7	.114	4.21
	2	1.74	.078	.0041	22.3	13.0	.083	1.81
	4	1.69	.073	.0018	23.1	13.4	.074	.67
3	1	1.89	.083	.0011	22.8	13.0	.145	3.51
	2	1.75	.076	.006	23.0	13.3	.089	1.46
	4	1.21	.052	.0011	23.2	13.4	.067	.75
4	1	1.91	.078	.005	24.5	13.0	.082	7.07
	2	1.80	.079	.005	22.7	13.2	.062	2.08
	4	1.40	.061	.002	22.9	13.3	.054	1.47
	Average of plots numbered 1	1.95	.084	.0024	23.3	12.8	.106	5.23
	Average of plots numbered 2	1.76	.078	.0041	22.5	13.1	.073	1.86
	Average of plots numbered 4	1.43	.061	.0017	22.8	13.4	.061	.97

[8] SCHOLLENBERGER, C. J., and DREIBELBIS, F. R. ANALYTICAL METHODS IN BASE EXCHANGE INVESTIGATIONS ON SOILS. Soil Sci. 30: 161–173, illus. 1930.

TABLE 5.—*Miscellaneous chemical and physical determinations on Leonardtown silt loam*—Continued

Range No.	Plot No.	Moisture equivalent	Colloid by mechanical analysis	Colloid by water-vapor absorption	Total base-exchange capacity per 100 g	Acidity per 100 g	Degree of saturation	pH
		Per cent	*Per cent*	*Per cent*	*Mg*	*Mg*	*Per cent*	
1	1	20.4	12.0	10.1	14.8	3.6	75.7	5.5
	2	20.0	11.9	9.6	14.3	3.36	76.5	5.8
	4	20.3	11.1	8.4	14.1	2.56	81.8	6.1
2	1	20.3	10.6	8.8	15.0	2.92	80.3	5.8
	2	20.4	11.7	8.7	14.4	1.82	87.2	6.9
	4	19.8	13.2	9.9	14.5	3.12	78.5	5.6
3	1	19.9	12.5	10.9	15.6	3.04	80.5	6.1
	2	19.8	11.6	9.2	14.0	2.80	80.0	5.8
	4	18.6	11.3	8.0	13.9	1.36	90.2	7.3
4	1	21.2	11.2	8.3	14.1	2.72	80.7	5.9
	2	21.1	12.3	9.6	13.7	2.40	82.5	5.7
	4	19.5	10.8	7.8	13.2	2.48	81.1	5.6
	Average of plots numbered 1	20.4	11.6	9.5	14.9	3.07	79.3	------
	Average of plots numbered 2	20.3	11.9	9.3	14.1	2.59	81.5	------
	Average of plots numbered 4	19.5	11.6	8.5	13.9	2.38	82.9	------

DISCUSSION

The mechanical analyses of the samples (Table 2), although they show some differences, on the whole bear out the assumption of uniform texture throughout the area of the plots. Some divergence is to be expected owing to the conditions existing in the soil at the time of collection. Moreover, as Table 5 shows, the variations in organic matter affect the degree of dispersion, since not all the organic matter is removed by hydrogen peroxide.

The analyses of the acid extract (Table 3) reveal the fact that in general the extracted components of the check plots are slightly less than the corresponding materials from the fertilized plots. This was to be expected, particularly in the case of calcium and phosphorus because of the large additions of these materials. The addition of calcium did not, however, make the calcium content of the fertilized plots greater than that of the check plot in range 3. That so large a quantity of calcium and magnesium was actually present in this check plot is shown not only by its exceptional pH value as compared with the pH values of the other check plots, but also by a determination of the calcium carbonate content. This latter value is 0.07 per cent for the check plot in range 3 as compared with 0.02, 0.03, and 0.05 per cent for the other check plots. These quantities were determined by the method outlined by Alexander and Byers.[9] The presence of calcium carbonate may be owing to the result of an undue share of the added crushed limestone previously mentioned. Some adventitious change in this plot is evident in other respects also, though the differences have not been adequate to alter its general yields materially. This increased solubility of the components of the fertilized plots is relatively much greater in the case of the phosphorus pentoxide. There is, however, but little difference between the effects produced by rock phosphate and superphosphate. The

[9] ALEXANDER, L. T., and BYERS, H. G. A CRITICAL LABORATORY REVIEW OF METHODS OF DETERMINING ORGANIC MATTER AND CARBONATES IN SOILS. U. S. Dept. Agr. Tech. Bul. 317, 26 p., illus. 1932.

soluble phosphorus pentoxide of plots 1 bears about the same ratio to that of plots 2 as the phosphorus pentoxide of the rock phosphates added to plots 1 bears to the phosphorus pentoxide of the superphosphate added to plots 2. The rock phosphate added to plots 1 is equivalent to about 1,000 pounds of phosphorus pentoxide an acre, whereas the superphosphate added to plots 2 is equivalent to about 175 pounds an acre. There is, however, but little difference between the crop yields of plots 1 and plots 2. This would indicate that more rock phosphate was added to plots 1 than was necessary. It seems evident, therefore, if we compare Tables 1 and 3 that the outstanding characteristic of this area, so far as inorganic constituents are concerned, is a deficiency in phosphates.

The increase in solubility of the aluminum in the fertilized plots is marked and again is greater in the plot 1 series than in the plot 2 series, and is, in all cases, greater than the check plots in the same range of plots. The writer has no explanation to offer for the relatively much greater solubility shown by all the plots in range 2 (in corn when the samples were taken) and range 3 (in soybeans). The alterations in the solubility of silica are not marked by the action of the fertilizer additions, but the ratio of alumina to silica (8.91) is of an order that indicates free alumina or the decomposition of colloid by the acid. There seems to be no explanation in the history of the soil treatment for the greater solubility of silica and alumina in ranges 2 and 3.

The general trend for manganese and for potassium is in the direction of increased solubility with the more effective fertilizer treatment, but the alteration, as shown by the averages, is not very strongly marked and in both cases may be due to absorption of these elements from the added manure.

The data given in Table 4 for calcium, magnesium, and potassium show the same general relationships as those given in Table 3. The quantity of sodium dissolved is apparently somewhat greater but does not reach values of any special significance. On the other hand, the manganese dissolved by the ammonium acetate is approximately one-fifth to one-seventh of that dissolved by the 0.05 normal acid. The aluminum dissolved is but one-twentieth of that soluble in acid, and the iron and phosphoric acid content of the dissolved material are reduced to a trace. Schollenberger and Dreibelbis believe that "any aluminum found in ammonium acetate extract of soil may be presumed to have entered the solution by true exchange." If this is true, it follows that exchangeable alumina in the small quantities present is not toxic, since the plot 1 series have a somewhat higher content and are notably more productive than the check plots.

In Table 5 are shown a number of determinations that are significant. The addition of the manure has markedly increased the organic content, as measured by the combustion method, of the plots to which it was added; yet the increase is apparently less when the manure is associated with superphosphate. The organic matter in the check plots remains considerable, even though no addition has occurred, certainly not during the 10-year observation period and possibly for a much longer time. Yet the organic matter-nitrogen ratio shows no appreciable alteration due to increase of organic content, and the carbon-nitrogen ratio also is almost constant. It follows from these observations that the nitrogen content alters with the organic content.

In the nitrate content of the soils the only definite trend observed is in the check plots, where the nitrate content is much lower, except in range 2, from which the corn crop has just been harvested. There is no apparent explanation of this variation since the plots were all very dry and had remained so for many weeks before sampling. The determination of the total phosphoric acid accounts fairly accurately for the total phosphates added to both series of plots 1 and 2. The percentage of the total phosphate soluble in acid is strikingly greater in the plots treated with rock phosphate and makes very apparent the probable cause of the variation in yields shown in Table 1.

The moisture-equivalent measurements show no sharply altered water-holding capacity in the fertilized and unfertilized plots and are of about the order of magnitude that would be expected from the differences in organic-matter content. The quantity of colloid in the plots, as shown by mechanical analysis (0.002 mm fraction), indicates no alteration, and the quantity differences, as shown by the water-vapor absorption method, are such as might be expected from the known difference of absorption values of organic and inorganic colloids. Similarly, the base-exchange capacity of the plots is that which would be expected from the organic-matter differences in them.

In general the total exchangeable acids of the fertilized plots vary with the pH and average higher than in the check plots. The higher value in the check plot of range 2 is undoubtedly due to the addition of the manure to this range in the spring of the year in which the sampling was made. As a result of the increased quantity of organic matter in the fertilized plots, the mean values of the degree of base saturation is lower than in the check plots. These latter values also indicate that the degree of base saturation is sufficient to maintain good crop conditions, and they tend to confirm the comment previously made that the deficiency in this soil which limits production is the small content of available phosphorus.

The range of pH values shown by the soils of these plots is narrow and apparently gives no indication of productivity.

SUMMARY AND CONCLUSIONS

This paper reports a study carried out for the purpose of ascertaining the chemical changes in the soil colloid of a given soil type resulting from a treatment which changed the soil from a condition of comparative nonproductivity to one capable of increased crop yields. The data presented indicate that the only apparently essential alteration consists in the supply of available phosphorus. Whether the coincident increase in organic matter has had a part in rendering the phosphate available or whether the organic supply in the check plots is adequate, is not clear from the analytical evidence. It is also true that the fertilizer treatment given the plots has increased the average available potassium, manganese, magnesium, and calcium, and the total nitrogen content of the treated plots. It is not clear, however, that these alterations are of great significance; in particular it appears that increase in the available calcium with the consequent change in pH value of the check plot of range 3 is not followed by increased productivity.

The results of this study, which are in accord with those obtained by Holmes in a study of the colloid of Leonardtown silt loam, make it

clear that the plant food present, with the exception of phosphorus, is adequate for good crop yields.

It appears from the data obtained that, although N/20 hydrochloric acid and normal ammonium acetate are essentially equally effective in the removal of exchangeable bases from this soil, the effect of these reagents on the sesquioxide, phosphoric acid, and silica are very different. It does not seem possible, from the data, to draw any conclusions regarding the ratios of plant-food materials which favor productivity.

OBSERVATIONS ON A SEVERE OUTBREAK OF MYCOSIS IN CHICKS[1]

By ERWIN L. JUNGHERR

Associate in Animal Pathology, Department of Animal Diseases, Connecticut (Storrs) Agricultural Experiment Station

INTRODUCTION

During the summer of 1931 the writer was called upon to investigate losses of chicks in a Connecticut hatchery that amounted to approximately 10,000 out of 50,000 hatched during the season, or about 20 per cent. This hatchery specialized in Single Comb White Leghorn pedigreed stock, and was in the process of eradicating pullorum disease according to the Connecticut official plan.

At first an unusual outbreak of bacillary white diarrhea was suspected, but detailed bacteriological examination of 45 chicks failed to reveal infection from this source. On further study the condition was found to be characterized by whitish lesions in the crop and by the presence of yeastlike fungi in the digestive tract. The disease was finally determined to be thrush, muguet (French), or Soorkrankheit (German).

LITERATURE

According to Kaupp,[2] chickens and turkeys affected with thrush, aphtha, or sore mouth appear dull and emaciated and often die in convulsions. The mucous lining of the mouth and esophagus shows whitish or brownish-yellow adherent deposits consisting of microscopic hyphae and oval spores of *Oidium* (*Saccharomyces*) *albicans*. Van Heelsbergen[3] calls the disease oidiomycosis, and describes it as an affection of the mucous membrane of the mouth, fauces, esophagus, crop, stomach, and small intestine of birds, mammals, and man. Young chickens, pigeons, geese, and turkeys appear to be especially susceptible. However, according to Lahaye, as quoted by Van Heelsbergen,[4] thrushlike affections in pigeons are often caused by fowl-pox virus. In the description of stomatitis oidica of birds, Hutyra and Marek[5] quote Schlegel who observed cases in which the mouth cavity showed only discoloration, while the glandular stomach and gizzard revealed true thrush mycosis or ulcerlike foci.

On the whole, a study of the available literature leaves the impression that the disease is of epidemiological importance in pigeons but not in chickens.

The disease reported here appears to be different from the mycosis of birds described by Staub and Truche,[6] for in the writer's cases the

[1] Received for publication Apr. 11, 1932, issued February, 1933.

[2] KAUPP, B. F. POULTRY DISEASES AND THEIR TREATMENT. Ed. 5, rev. and enl., 436 p., illus. Chicago. 1929.

[3] HEELSBERGEN, T. VAN. HANDBUCH DER GEFLÜGELKRANKHEITEN UND DER GEFLÜGELZUCHT. 608 p., illus. Stuttgart. 1929.

[4] HEELSBERGEN, T. VAN. Op. cit.

[5] HUTYRA, F., and MAREK, J. SPECIAL PATHOLOGY AND THERAPEUTICS OF THE DISEASES OF DOMESTIC ANIMALS. 3d authorized Amer. ed. from 6th rev. and enl. German ed., 3 v., illus. Chicago. 1926.

[6] STAUB, A., and TRUCHE, C. THE FIGHT AGAINST INFECTIOUS DISEASES OF POULTRY IN FRANCE. 4th World's Poultry Cong. Rpt. 1930 (Sec. C): 386-388. 1931.

heart, liver, and peritoneum did not show the typical whitish films. It likewise differed from mycosis of turkeys, said by some to be identical with oidiomycosis of chickens.[7] The writer[8] has found the former disease to be characterized by yellow, conical, slightly curved processes with a hemorrhagic areola. These young lesions may become confluent and form extensive ulcers in the crop, esophagus, and glandular stomach. Such pathological manifestations, aside from the unknown etiology of mycosis in turkeys, constitute, in the writer's opinion, a distinction between thrushlike affections of turkeys and chickens.

EPIDEMOLOGY

The plant in which the present observations were carried out was situated on a well-drained hilly plateau and spread out over considerable territory. Brooding was carried on in 9 by 12 foot colony houses which were kept rather warm during the summer. The disease first appeared in the second or third hatch of the season,

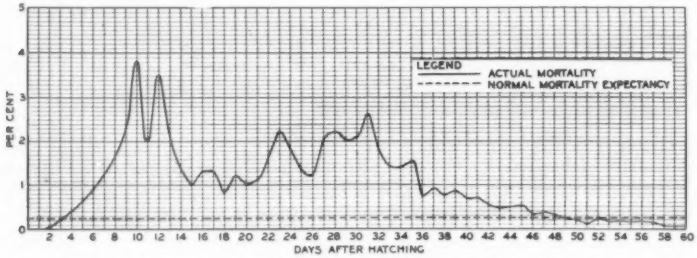


FIGURE 1.—Average daily mortality of 4,919 chicks during the first 60 days after hatching as compared with normal mortality expectancy

during the middle of April, increased in intensity during June and July, and disappeared at the end of August.

Clinical symptoms appeared from six to seven days after hatching and consisted of dullness, listlessness, huddling together, and marked roughness of the feathers. Gaping and diarrhea were not noticed as flock symptoms. Mortality started almost simultaneously with the appearance of clinical evidence, and reached a peak about the tenth day and again about the thirty-first day after hatching. Chicks that reached the age of 60 days had apparently recovered from the disease and developed into normal birds.

Daily records were kept of the mortality in 10 colony houses having a population of 4,919 chicks. From these records the total daily mortality was computed in terms of percentage and the data plotted as shown in Figure 1. For comparison, a corresponding line was plotted, based on a normally expected mortality of 15 per cent during the first 60 days after hatching. This latter figure was taken as the high average of a 3-year chick-mortality survey carried out in Connecticut.[9]

[7] HUTYRA, F., and MAREK, J. Op. cit. KAUPP, B. F. Op. cit. HEELSBERGEN, T. VAN. Op. cit.

[8] JUNGHERR, E. TWO INTERESTING TURKEY DISEASES. Jour. Amer. Vet. Med. Assoc. 71: 636-641, illus. 1927.

[9] JONES, R. E. THREE YEARS' GROW HEALTHY CHICKS SURVEY FIGURES. Conn. Agr. Ext. Serv., Home Egg Laying Contest v. 10, 1st issue November. 1928.

The mortality in the 10 colony houses under observation varied considerably, as is shown in Figure 2. The thin lines represent individual colony houses, the numbers indicating the population. The heavily stocked colony houses appear to have suffered most, and overcrowding may therefore be considered a factor in the spread of the

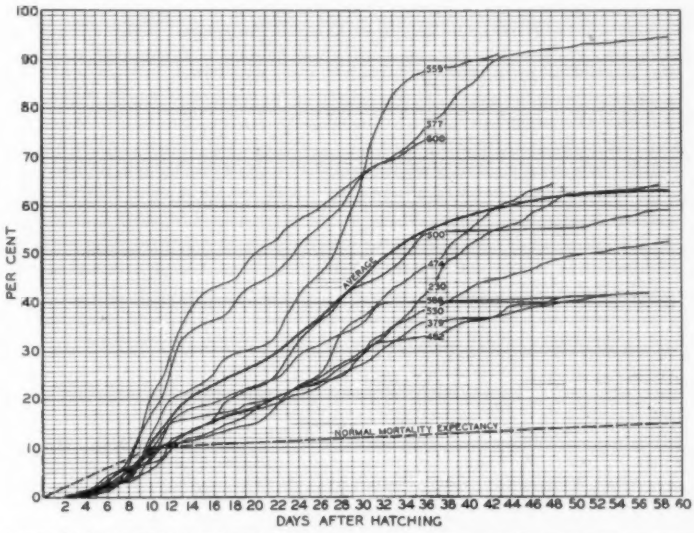


FIGURE 2.—Mortality of 4,919 chicks in 10 colony houses during the first 60 days after hatching, as compared with normal mortality expectancy; numbers indicate population of colony houses; heavy line shows total average mortality

disease. The thick line represents a total average mortality of 63 per cent.

During the ravages of the disease various changes in management were made in an attempt to check the losses. The results are shown in Table 1.

TABLE 1.—*Comparison of loss of chicks reared under different conditions*

Point of comparison	Condition of rearing	Number of chicks	Number died	Per cent of loss during first 60 days of life
Eggs and incubators	Native [a] eggs hatched in native incubators	3,858	2,606	67.5
	Native eggs hatched in foreign [a] incubators	760	424	55.8
	Foreign eggs hatched in foreign incubators	302	99	32.8
Sanitation in colony house	Chicks in colony houses cleaned once a week	559	514	91.8
	Chicks in colony house cleaned once a day	588	243	41.3
	Chicks in colony house on wire floors	482	202	41.9

[a] Native=on the original hatchery plant; foreign=away from the original hatchery plant.

It will be seen that a change in incubators brought about a slight decrease in mortality, but certainly not enough for all the losses to be attributed to incubation. Even chicks from an entirely different

source became affected by the disease when brought to the premises, suffered a loss of 32.8 per cent. Neither a daily cleaning of the colony houses nor the use of wire floors was very effective in controlling the disease, for the losses under strictly sanitary conditions amounted to 41.9 per cent.

The total actual mortality in this outbreak can only be estimated, since about 90 per cent of the total output of 50,000 was sold as day-old chicks. According to the original owner, the purchasers reported losses aggregating 6,000, under circumstances very closely resembling those observed on the home plant. While there is no direct evidence that all of these 6,000 chicks died from the same cause, the writer found the characteristic lesions of thrush in the organs of five abnormal chicks selected at random from a group that had been purchased from the home plant. The losses were reported principally by buyers of large lots; the buyers of small lots, or rather those who brooded the chicks in small flocks or batteries, apparently had better luck. The widespread reports of losses would indicate that feeding methods, which were known to be quite varied among the owners, could not be considered an important factor in the trouble.

PATHOLOGY

In a study of the disease it was surprising to find that gross lesions in chicks from 2 to 3 weeks old were often so small as not to be easily distinguishable. In older chicks gross lesions tend to become localized in the crop and are readily recognized. In 120 field cases recorded, 84 showed lesions in the crop, 29 in the proventriculus, 53 in the gizzard, 6 in the liver, and 44 in the gall bladder; in 12 field cases no macroscopic lesions were observed.

In very young chicks the gross lesions in the crop consisted of pinhead-sized whitish nodules, and in older chicks of circular whitish ulcerlike patches (fig. 3, A) ranging in diameter from 1 to 5 mm., or of grayish-white pseudomembranes often forming a ridge along the folds (fig. 3, B). Very often only loose necrotic material with no apparent injury to the underlying tissue was found. As a rule, the unopened crop appeared tympanitic on palpation.

A microscopic examination of the crop showed extensive destruction of the stratified epithelium deep in the Malpighian layer (fig. 3, C), and quite often walled-off ulcers (fig. 3, D) or extensive diphtheroid to diptheritic membranes. The lesions were characterized by the absence of inflammatory reaction.

The proventricular wall appeared at times diffusely swollen, or exhibited whitish, opaque, circular areas on the serous surface. The mucous surface was covered with brownish or mucoid exudate. Under the microscope necrotic lesions were seen in the epithelium of the superficial glands and in the centers of small lymph nodes. (Fig. 4, A and B.)

The corneous lining of the gizzard frequently revealed ulcers (fig. 4, C), especially in the cardiac region (fig. 4, D), or old hemorrhages which were visible by transmitted light (fig. 4, E). The true mucous membrane of the gizzard had usually lost its normal glossiness and appeared opaque and grayish white, or it showed whitish shallow ulcers. On microscopic examination, the ulcers in the lining were found to consist mostly of craters in the tissue, with little cellular reaction around them.

In the majority of cases the liver was normal, although occasionally whitish pinhead-sized nodules or small hemorrhagic infarcts were seen. Sections of some livers which appeared normal to the naked eye showed, under the microscope, periportal focal necrosis. (Fig. 5,

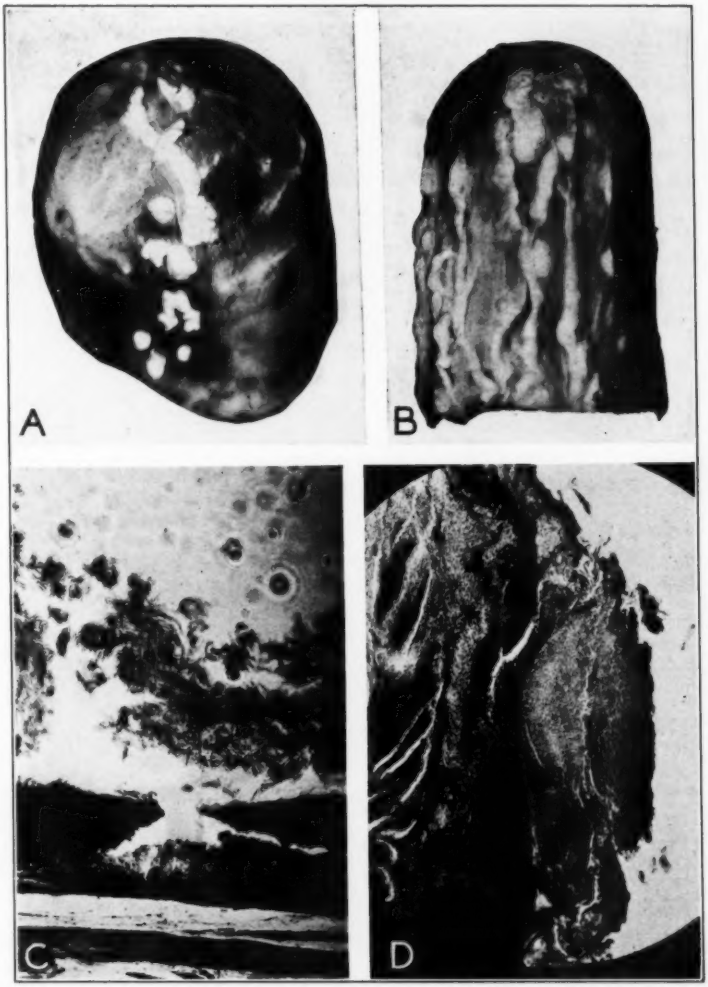


FIGURE 3.—A, Inverted crop No. 1513, showing circular mycotic lesions, × 3; B, inverted crop No. 1488, showing mycotic lesions along the folds, × 3; C, section of crop No. 1500, showing extensive destruction of stratified epithelium, × 100; D, section of crop No. 1642, showing walled-off ulcer, × 70

A.) The gall bladder was often enlarged and the bile thickened. The duodenum appeared congested and hemorrhagic, and the caecal contents foamy. In some instances the caeca contained large numbers of a protozoon which morphologically resembled *Trichomonas*

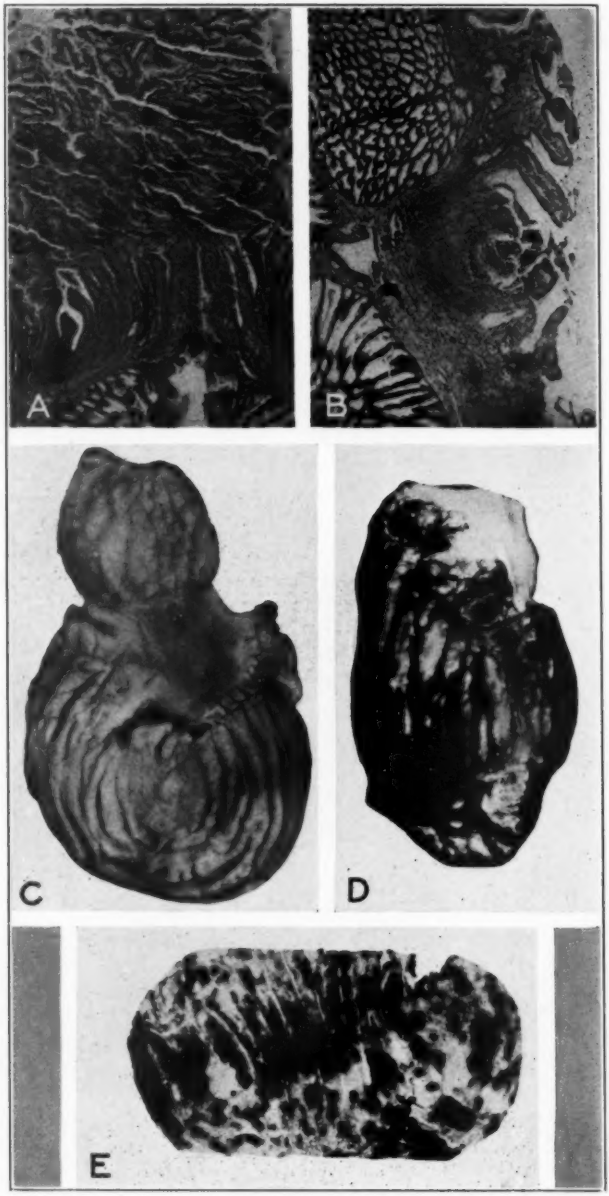


FIGURE 4.—A, Section of proventriculus No. 1732, showing necrosis of epithelium of superficial glands, × 70; B, section of proventriculus No. 1731, showing focal necrosis of lymph nodule, × 70; C, inverted gizzard No. 1596, showing superficial ulcer in corneous lining, × 2; D, inverted gizzard No. 1673, showing circular ulcers at cardia, × 2; E, corneous lining of gizzard No. 1772, photographed by transmitted light, and showing old hemorrhages and whitish, shallow ulcers, × 1.5

eberthi; occasionally they contained hemorrhagic cores which were probably due to an intercurrent infection with *Eimeria tennella.* The kidneys often appeared pale and slightly tumefied. The ureters were distended.

ETIOLOGY

In the literature [10] the statement is frequently made that hyphae can be demonstrated readily in scrapings of lesions. In the present study mycelial threads were found infrequently, but spores could be seen, although recognition of them as such was attended with some difficulty.

Since cultural studies on routine laboratory media were inconclusive, the use of Sabouraud agar was resorted to. From 64 field cases that were cultured yeastlike fungi were isolated in 54 instances from various organs, such as the crop, proventriculus, gizzard lining, true mucous membrane of the gizzard, the liver, gall bladder, and intestine. As a rule, only one type of organism was isolated from any one organ.

The types of yeastlike fungi isolated fall into three main groups, according to their growth characteristics on Sabouraud agar. The first type, which was isolated 38 times, produced a dry, whitish, high convex colony, consisting of spherical conidia with little or no mycelial structures in young cultures. This organism produced general infection in rabbits, on intravenous injection, and according to Benham [11] resembled *Monilia albicans.* The second type, which was isolated 16 times, developed a rough, feathery, adherent colony, consisting of thick mycelium and spores. It was not pathogenic to rabbits and resembled *Oidium lactis.* The third type, isolated fifteen times, produced a smooth, grayish, flat colony, consisting of small elliptical conidia. It was not pathogenic to rabbits and resembled *Monilia krusei.*

In an effort to reproduce the disease by artificial infection, tests were made with 52 chicks ranging in age from 3 to 22 days. Five chicks were fed with a composite fecal specimen from field cases. Four of the chicks succumbed to the infection. (Table 2.) All showed typical lesions in the crop (fig. 5, B) and one of them in the mouth, also. This was the only mouth lesion seen during the outbreak. *Monilia albicans* was isolated from the principal lesions. This experiment proved rather conclusively that droppings from diseased birds are infectious and that *M. albicans* must be considered the principal cause of the disorder. To test the pathogenicity of pure cultures, 47 of the 52 chicks were injected in different ways. *M. albicans* was found to be highly pathogenic, *Oidium lactis* much less so, and *M. krusei* was apparently not harmful under experimental conditions. (Table 2.)

The method of administration, whether oral, subcutaneous, intravenous, or intraperitoneal, did not seem to be of particular importance in the demonstration of pathogenicity, but the age of the chick appeared to be a definite factor, chicks from 1 to 2 weeks old being most susceptible.

[10] HUTYRA, F., and MAREK, J. Op. cit. KAUPP, B. F. Op. cit. HEELSBERGEN, T. VAN. Op. cit.
[11] BENHAM, R. W. Private communication. 1931.

In these experiments the time from artificial infection to death ranged from 10 to 45 days, the average being 31 days. It should be kept in mind, however, that these experiments took into account

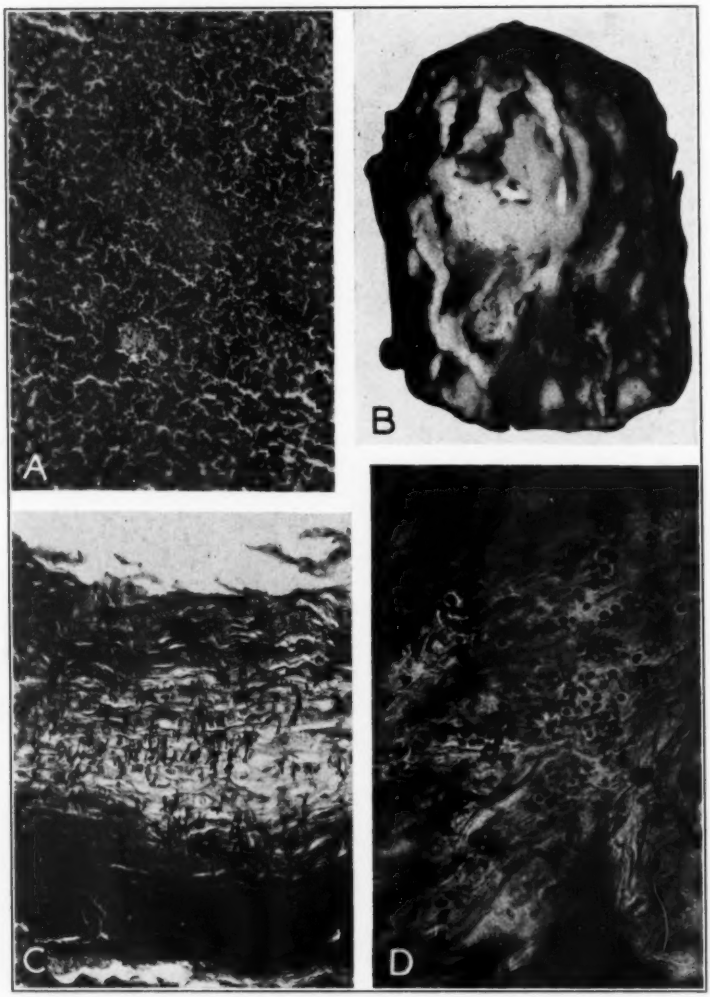


FIGURE 5.—A, Section of liver No. 1724, showing focal necrotic areas, × 140; B, inverted crop No. 1586, showing lesions produced in chick by feeding fecal material from infected birds, × 3; C, section of crop No. 1621, showing numerous mycelial structures in stratified epithelium, × 200; D, section of crop No. 1488, showing clusters of spores in stratified epithelium, × 600

only actual mortality and not morbidity, which is known from field observations to be always considerably higher.

In a further study of the mode of transmission of the disease 100 eggs were hatched in sterilized incubators and the chicks reared in

sterilized battery brooders. In the crops of four of these chicks characteristic lesions of thrush were found and *Monilia albicans* isolated from them. Some of the hens from the poultry plant suffered from an extremely moist type of vent gleet, and a mycological examination of lesions from these birds yielded fungi of the *M. albicans* type.

TABLE 2.—*Results of experimental feedings of or injections with infectious material*

Infectious material	Number of chicks infected	Fatal cases
Feces	5	4
Pure cultures resembling—		
Monilia albicans	16	11
Oidium lactis	16	2
Monilia krusei	15	0

As a final link in the evidence of the etiological relationship of yeastlike fungi to the disease outbreak, mycelial structures (figs. 3, C, and 5, C), or clusters of spores (fig. 5, D), were often demonstrated in microscopic sections of the organs.

DISCUSSION AND SUMMARY

Losses totaling 10,000 chicks were observed in a commercial hatchery of 50,000 chicks that were less than 60 days old. The average loss in the home plant was 63 per cent, and in the chicks that were sold as day old, 13 per cent. The morbidity was much higher, according to the clinical appearance of the flocks. The chicks that survived made good recovery, and 60 days after hatching had developed into normal birds. Losses were smaller when brooding was carried out in small lots or batteries; change in feed and rigid sanitary precautions were not very effective as control measures.

The diseased condition was characterized by whitish ulcers or pseudomembranes in the crop, brownish or mucoid deposits in the proventriculus, and ulcers in the gizzard. The lesions in small chicks were often so small as to be easily overlooked.

On mycological examination yeastlike fungi were isolated from the crop, proventriculus, gizzard, gall bladder, and intestine. The predominating organism was a yeastlike fungus resembling *Monilia albicans*; the other observed types resembled *Oidium lactis* and *M. krusei*.

The diseased condition was reproduced by feeding fecal material from diseased chicks and by injecting fungus cultures of the *Monilia albicans* type. In two instances artificially infected chicks succumbed with the *Oidium lactis* type. Infection experiments conducted on small lots of chicks were not uniformly successful, a point readily understood when field observations of small-lot brooding are considered. The average period of incubation under experimental conditions was 31 days, a period that closely coincided with the second mortality peak under field conditions. The second mortality peak may, therefore, have been due to postnatal infection.

The disease may appear in septicaemic form, as was indicated by the isolation of virulent fungi from the liver and gall bladder; how-

ever, the extreme virulence of the infection in very young chicks, together with the slight pathological changes that occurred and the occasional presence of focal necrosis in the liver, suggests a toxemic action of the fungi.

Day-old chicks from the hatchery developed the disease after they had been removed from the original premises. Chicks raised on wire floors in a new, isolated colony house suffered 41.9 per cent losses. The first and highest mortality peak occurred about the tenth day after hatching; the average incubation period under experimental conditions was 31 days. The cloaca of laying hens affected with a moist type of vent gleet was shown to carry yeastlike fungi. When eggs from the plant were hatched in a sterilized incubator and the chicks reared in sterilized battery brooders, the disease appeared in only a few instances. These data furnish circumstantial evidence that the disease can be transmitted through the agency of the egg, presumably on the egg.

The fact that the owner found that brooding in batteries was safer than in colony houses, and the writer's observation that only relatively small numbers of chicks succumbed to experimental infection, suggest that management may play a part in the postnatal spread of the disease.

According to available information, this report of a severe outbreak of thrush or mycosis in chicks is the first in which the disease has been shown to be of major epidemiological importance.

THE EFFECT OF DIFFERENT SOAPS ON LEAD ARSENATE IN SPRAY MIXTURES [1]

By JOSEPH M. GINSBURG

Biochemist in Entomological Investigations, New Jersey Agricultural Experiment Station

INTRODUCTION

Among the various chemicals employed to increase the spreading properties of insecticides on foliage, soaps undoubtedly rank as the most efficient and cheapest. Yet soaps can not be safely used with sprays containing arsenicals because the chemical reaction involved in such mixtures tends to liberate soluble arsenic, often in quantities large enough to produce injury to the foliage. It has been shown by Tartar and Bundy [2] and Pinckney [3] that acid lead arsenate ($PbHAsO_4$) produces much more soluble arsenic than the basic lead arsenate ($Pb_5OH(AsO_4)_3$) when mixed with soaps. Pinckney has also shown that a stearate soap forms considerably more soluble arsenic than does an oleate soap when mixed with either the basic or the acid lead arsenate.

The differences observed between the two fatty acids mentioned above suggested that soaps consisting of the same fatty acid but of different bases might exhibit similar or even greater differences in their action on lead arsenate. Thus, soaps having a strong base, such as sodium or potassium, might produce more soluble arsenic and consequently greater injury to foliage than soaps having a weak base, such as ammonium, triethanolamine, etc. If such should be the case it might be possible to prepare a soap from oleic acid and a very weak base which would be compatible with lead arsenate spray mixtures and safe to use on foliage. Accordingly, soaps of different bases were tested in field and laboratory experiments.

FIELD TESTS

During July, 1930, several apple trees were sprayed with mixtures consisting of acid lead arsenate, hydrated ferric oxide, and soap. The proportions of the ingredient per 100 gallons of spray were as follows: 3 pounds lead arsenate, 4 pounds ferric oxide, and 0.5 per cent soap. In previous tests it had been found that this amount of soap was sufficient to produce good spreading of the lead arsenate on apple foliage. The ferric oxide was added as a sticker and corrective. The ability of this chemical to increase the adhesion of lead arsenate and prevent arsenical injury to foliage has already been shown.[4] The three soaps selected for the test were commercial potash fish-oil soap, potassium oleate, and triethanolamine oleate. The last two soaps

[1] Received for publication Apr. 6, 1932; issued February, 1933. Paper of the Journal Series, New Jersey Agricultural Experiment Station, Department of Entomology.

[2] TARTAR, H. V., and BUNDY, L. A. SOLUBLE ARSENIC IN MIXTURES OF LEAD ARSENATE AND SOAP, Jour. Indus. and Engin. Chem. 5: 561–562. 1913.

[3] PINCKNEY, R. M. ACTION OF SOAP UPON LEAD ARSENATES. Jour. Agr. Research 24: 87–95. 1923.

[4] GINSBURG, J. M., and MANN, R. F. STUDIES WITH HYDRATED FERRIC OXIDE AS CORRECTIVE AND STICKER FOR LEAD ARSENATE AND NICOTINE TANNATE. Jour. Econ. Ent. 24: 695–701. 1931.

were prepared in the laboratory from pure chemicals. The first two soaps contain a strong, highly dissociating inorganic base (K), while the third contains the comparatively weak organic base $(CH_2CH_2OH)_3N$. The ingredients were mixed in the following manner: The lead arsenate and ferric oxide were first made into a thin paste by the addition of a small amount of water. The rest of the water containing the required amount of soap was then added. The spray was applied from a 3-gallon bucket pump. The trees received three sprays at 2-week intervals.

TABLE 1.—*Results of spraying apple trees with lead arsenate and ferric oxide, with different soaps as spreaders, 1930*

Soap used	Injury observed on trees sprayed—			Nature of injury
	July 3	July 17	July 30	
Potassium oleate	Very slight	Slight	Appreciable [a]	Brown leaf areas.
Triethanolamine oleate	None	None	Slight [a]	A few brown leaf tips.
Potash fish-oil soap (commercial).	Appreciable	Severe	Severe [a]	About 30 per cent defoliation
Check (no soap)	None	None	None [a]	

[a] Observations made on Aug. 10.

The results, which are presented in Table 1, show that arsenical injury from the sprays containing either fish-oil soap or potassium oleate was apparent after the first application. The injury from fish-oil soap was more pronounced and increased severely with each subsequent spraying, until by August 10 there was about 30 per cent leaf drop. The potassium oleate produced brown spots on the leaves but did not cause defoliation. The trees sprayed with triethanolamine oleate showed no injury until after the third application and even then injury was slight as compared with that produced by the potassium soaps. There was no visible arsenical injury on the check trees sprayed with lead arsenate and ferric oxide only.

LABORATORY TESTS

Soaps were prepared from pure oleic acid with the following bases: Sodium, potassium, ammonium, and triethanolamine $(CH_2CH_2OH)_3N$. In addition to these pure soaps, a commercial fish-oil soap was used. Samples of acid lead arsenate in concentrations equivalent to 3 pounds in 100 gallons of spray were mixed in flasks containing 500 c c of distilled water with various dilutions of soaps (0.25, 0.5, 1 per cent) and allowed to stand for 24 hours, with frequent shaking. At the end of this period the soluble arsenic was determined by the procedure outlined in the official methods of the Association of Official Agricultural Chemists.[5] The only deviation from this method was that the mixtures were kept at room temperature instead of at 32° C. The lead arsenate was a commercial brand, which contained 32.4 per cent As_2O_5.

[5] ASSOCIATION OF OFFICIAL AGRICULTURAL CHEMISTS. OFFICIAL AND TENTATIVE METHODS OF ANALYSIS. COMPILED BY THE COMMITTEE ON EDITING METHODS OF ANALYSIS. REVISED TO JULY 1, 1924. Ed. 2, 535 p., illus. Washington, D. C. 1925.

TABLE 2.—*Soluble arsenic liberated from 1.8 g lead arsenate mixed in 500 c c of water with various concentrations of soaps*

Soap used	Concentration of soap	Soluble As_2O_3 formed	Lead arsenate in solution
	Per cent	*Per cent*	*Per cent*
Potash fish-oil soap (commercial)	0.25	13.32	47.28
	.50	20.87	74.10
	1.00	21.56	76.50
Sodium oleate	.25	9.25	32.83
	.50	10.80	38.34
	1.00	16.79	59.60
Potassium oleate	.25	8.15	28.93
	.50	9.78	34.71
	1.00	10.94	38.83
Ammonium oleate	.25	5.39	19.13
	.50	6.67	22.78
	1.00	6.14	21.80
Triethanolamine oleate	.25	3.83	13.59
	.50	4.63	16.44
	1.00	5.59	19.84
Check (lead arsenate only)		.43	1.53

The results, presented in Table 2, clearly show that soaps of strong bases liberate more soluble arsenic than soaps of weak bases. Thus, ammonium oleate gave considerably less soluble arsenic than the oleate of either potassium or sodium, while the triethanolamine oleate, the weakest base tested in this experiment, gave the lowest amount of soluble arsenic.

A comparison of the results in Table 2 brings out the fact that the amount of lead arsenate decomposed is not in direct proportion to the amount of soap present in solution. Thus, 0.25 per cent potassium oleate decomposed 28.93 per cent lead arsenate, while 1 per cent of the same soap decomposed only 38.83 per cent. Again, 0.25 per cent ammonium oleate dissolved 19.13 per cent, of the lead arsenate, while 1 per cent ammonium oleate dissolved only 21.80 per cent. Evidently the reaction does not go to completion and can not be represented by the simple formula

$$PbHAsO_4 + {}^2C_{17}H_{33}COOK = K_2HAsO_4 + (C_{17}H_{33}COO)_3Pb$$

The reaction is more probably of double decomposition, as suggested by Smith.[6]

$$5PbHAsO_4 + 4C_{17}H_{33}COOK + H_2O = Pb_5OH(AsO_4)_3 + 4C_{17}H_{33}COOH + 2K_2HAsO_4$$

The results from the laboratory tests fully explain why considerably more injury to apple foliage resulted from arsenical mixtures containing potassium oleate than from those containing triethanolamine soap. The former produced more than twice as much soluble arsenic as did the latter. These results further explain why most of the foliage injury was produced by the spray containing fish-oil soap. This soap decomposed more lead arsenate than any one of the other soaps. Evidently the amount of hydrated ferric oxide used in the field tests was not sufficient to take care of the large quantities of soluble arsenic liberated from the lead arsenate by the fish-oil soap. It is clear from

[6] SMITH, G. E. LEAD ARSENATES. A STUDY OF THE FACTORS CONTROLLING THE REACTIONS OF LEAD NITRATE AND LEAD ACETATE WITH DISODIUM ARSENATE. Jour. Amer. Chem. Soc. 38: 2014-2027. 1916.

the results here shown that soaps, if used at all with lead arsenate, should not consist of a mixture of fatty acids, such as those present in fish oil, but should be prepared from pure oleic acid and a weak base.

SUMMARY OF RESULTS

Apple trees were sprayed three times at 2-week intervals with mixtures consisting of lead arsenate, hydrated ferric oxide, and each of the following soaps: Commercial potash fish-oil soap, potassium oleate, and triethanolamine oleate.

The spray containing fish-oil soap produced severe injury and caused about 30 per cent leaf drop. The injury from the potassium oleate spray was limited to spots on the leaves and browning of the leaf edges. The injury from the triethanolamine oleate spray was very slight and did not appear until after the third application.

Simultaneously with the field tests the action of the following soaps on lead arsenate was tested in the laboratory: Potash fish-oil soap, sodium oleate, potassium oleate, ammonium oleate, and triethanolamine oleate. The results indicate that soaps of strong bases form more soluble arsenic than soaps of weak bases. Of all the soaps tested, potash fish-oil soap produced the greatest quantity of soluble arsenic and triethanolamine oleate the least.

A METHOD FOR DETERMINING THE SPECIFICITY OF THE INTRACELLULAR GLOBULIN OF FUSARIUM LINI[1]

By CASPER I. NELSON

Bacteriologist and Soil Biologist, North Dakota Agricultural Experiment Station

INTRODUCTION

Groups and species of plants and animals have been successfully differentiated by serological means through the use of protein fractions from these organisms. The success of such methods of differentiation depends in part upon the natural specificity of the protein obtained and the sensitiveness of the technic used in demonstrating this specificity. Such technic, while it has not received universal sanction, has proved successful in some practical ways. Success with this type of work will be more common when the methods devised are less destructive of specific factors which are characteristic of the protein fractions. Globulins and other specific protein fractions are extremely sensitive to alteration, whether it be imposed by laboratory practice or by physiological factors, such as disease and disturbed metabolism. Indications of these alterations are numerous in syphilology and animal pathology. There are indications that variations in globulin may accompany disease even in plants (*5*).[2]

By means of direct and cross titrations of antisera produced with globulin antigens from various varieties of flaxseed it has been possible to differentiate the flax strains on the basis of wilt resistance (*8*). By analogous means it has been possible to classify various wheat hybrids relative to their interrelationships (*7*). In both cases protein fractions corresponding to the so-called globulins obtainable from antigenic materials were used to produce the antisera for the titrations.

THE PROBLEM

Varieties of flax resistant to the wilt organism (*Fusarium lini* Bolley) in one locality have not proved equally resistant in other localities. Frequently these localities are not sufficiently dissimilar for the differences in resistance to be explained on the basis of climate alone. Broadfoot (*1*) has suggested that they may be due to physiological variations in the parasitic fungus. If this is the case, physiologic strains must be characterized by more than morphologic factors—presumably by biochemical ones.

The author has never found described in the literature a satisfactory method for studying the protein content of fungus material. It is generally stated that mycelium is meagerly supplied with protein. Heck (*6*) suggests a ratio of carbon to nitrogen of about 7 or 10 to 1. This ratio is largely determined by the composition of the culture medium. The usual methods fail to demonstrate the full protein content of the mycelium. Before physiologic strains of fungi can be differentiated through the use of intracellular proteins, a method for

[1] Received for publication Apr. 11, 1932; issued February, 1933.

[2] Reference is made by number (italic) to Literature Cited, p. 187.

obtaining these proteins must be developed, and their specific antigenic nature demonstrated. A method suitable for such a purpose is presented in this report.

CULTURES

The cultures used were strains of *Fusarium lini* which had been isolated at the University of Minnesota. These were grown on hard potato agar and on corn-meal agar. All transfers were made in an inoculation room; stock transfers were made with a bit of mycelium, but for general purposes a cubic centimeter of young culture was drawn from a flask with a sterile pipette and used to inoculate liquid media. Erlenmeyer flasks of 250 c c capacity were used for culturing the fungus. The incubation temperature was about 28° C.

It was difficult to obtain a satisfactory type of growth in a reasonable length of time, that is, in three to four weeks. In trying to do this, various synthetic media were used, such as Czapek's sucrose nitrate solution, Richards' solution, peptone broth, and Raulin's solution. Of these, Raulin's solution gave the most satisfactory growth.

The most important consideration in this work was the amount of carbohydrate available in the medium. If too little was present the growth was feeble and yielded little protein, if too much the fungus rapidly reached maturity and converted the excess carbohydrate into reserve stores of fat. Such synthesis of fats was followed by a considerable lag in the growth.

Growth on Raulin's solution consisted of mats of mycelium and spores. Three types of spores were observed, microspores, macrospores, and chlamydospores. Of these the most numerous were the microspores. Young growth 2 to 3 weeks old was used, since it was found that such material was more easily extracted and gave a better yield of protein than similar amounts of older material. Young material was relatively free from storage fat, which could always be detected in older cultures, even without an excess of carbohydrate.

EXTRACTION OF PROTEIN

Two methods of extraction were developed. (1) The mycelial mats were drained and then ground in a ball mill for from 36 to 48 hours in a 5 per cent solution of sodium chloride. The macerated mass was centrifuged at 3,000 revolutions per minute for 10 to 20 minutes, and the opaque liquid was then electrodialyzed in a Bronfenbrenner apparatus. The resulting precipitate gave a very weak biuret reaction. The mycelial residue, alkalinized with N/100 NaOH gave a strong biuret reaction, indicating that the method had not satisfactorily extracted the protein fraction from the mycelial mass.

(2) A method suggested by the work of Chibnall (*2, 3, 4*) was next used. Mats of mycelium 3 to 4 weeks old were drained and dried for 3 to 4 hours at 35° C. in an incubator room. They were then immersed in ether for 1½ to 2 minutes. Ether was removed by evaporation in the incubator room for 5 to 6 hours. The mycelial mass was then placed in muslin sacks and subjected to heavy pressure in a screw press. A clear brownish liquid was expressed which gave a strong positive biuret reaction. According to Chibnall, this liquid is called "cell sap." It was electrodialyzed at between 20° and 30°, the process taking from 1½ to 4 hours. Before electrodialysis, the pH

value of this cell sap was 5.6; after dialysis it was 7.4 to 8. The precipitated material in the electrodialyzer, presumably now an isoelectric globulin, was held in very fine suspension, necessitating concentration in a centrifuge. The solid materials thus obtained were dissolved in 1 per cent sodium chloride. This solution was used for sensitization of rabbits and also as an antigen for precipitin titrations. The mycelial residue left after pressing was ground in the ball mill for 36 hours with 1 per cent sodium chloride. This material without further purification or electrodialysis was also used for sensitizing rabbits. All materials were stored in a refrigerator during the progress of the experiment.

SENSITIZATION OF ANIMALS

The half-grown rabbits used had never been fed flax. They showed very little variation in weight during the period of sensitization. As stated above, two types of antigen solution were used, namely, purified globulin, in 1 per cent saline, and mycelial residue, in 1 per cent saline. The purified globulin in 0.5 c c to 1.0 c c amounts was injected at 1 to 5 day intervals, the smaller amount being given at shorter intervals than the larger amounts. The mycelial residue was injected in 0.25 c c to 1 c c amounts. All injections were intraperitoneal. The total period of injections was about 20 days. No local infection resulted from either type of injection. A strong Arthus's phenomenon was observed in connection with mycelium when this matter escaped subcutaneously. Absorption of such material was evidently very slow.

SERUM TITRATIONS

Five days after the last injection 15 c c of blood was obtained from the sensitized rabbits and checks by slitting a vein in the ear with a small sterile blade. The blood was kept in the refrigerator overnight and the serum was then separated from the clot and dilutions made in a 0.9 per cent sodium chloride solution as indicated in titrations. The concentration of antigen was held uniform.

In the first experiment the quantity of available antigenic material (purified globulin) was so small that all titrations were made in test tubes of 0.25 c c capacity. The total volume used in each tube was 0.04 c c made up of 0.02 c c of antigen and 0.02 c c of serum in different dilutions. Precipitates were observed by using a lens of 10 × magnification. Characteristic mealy gray precipitates were obtained.

As shown in Tables 1 and 2, spores of *Fusarium lini* did not agglutinate when either sera in several dilutions was added to a suspension of the spores and observed microscopically. The quantitative estimation of agglutination was not possible since the spores precipitated by gravity.

TABLE 1.—*Antigens versus homologous antiserums, using microtechnic* [1]

Antigen	Precipitate at serum dilutions of—			
	1:20	1:40	1:80	1:160
Mycelium	+	+	+	++
Globulin	++	+++	+++	++++

[1] Plus signs have been used to indicate the quantity of precipitate as a measure of degree of specificity.

TABLE 2.—*Mycelium antiserum and globulin antiserum versus mycelium antigen, using microtechnic* [1]

Antiserum	Precipitate at serum dilution of—						
	1:20	1:40	1:80	1:160	1:320	1:400	1:640
Mycelium	+	++	++	++	+	0	0
Globulin	+++	++	++	++	0	0	0

[1] Plus signs have been used to indicate the quantity of precipitate as a measure of degree of specificity.

When a larger supply of antigen became available titrations were repeated. In these titrations the total volume of liquid in each test tube was 1 c c, made up of 0.5 c c of antigen and 0.5 c c of serum dilution. The results (Table 3) agree with those obtained in the first experiment in which minute amounts of reagent materials were used.

TABLE 3.—*Mycelium antiserum and globulin antiserum versus mycelium antigen, using larger volumes* [1]

Antiserum	Precipitate at serum dilution of—						
	1:20	1:40	1:80	1:160	1:320	1:400	1:640
Mycelium	------	+	+	+	0	------	------
Globulin	------	++	+++	+++	++	------	+
Normal serum	0	0	0	0	0	0	0

[1] Plus signs have been used to indicate the quantity of precipitate as a measure of degree, of specificity.

A comparison of Table 3 with Tables 1 and 2 shows no superiority in titration results where larger amounts of material were used.

The tables indicate that: (1) Both globulin and the mycelial residue from which the globulin was obtained possess specific antigenic properties. (2) Globulin is a more reactive antigen than the mycelial residue in homologous titrations. (Table 1.) (3) Globulin is a more reactive antigen than mycelial residue even when its antiserum is titrated against the heterologous antigen mycelial residue. (Table 2.) (4) When titrations were repeated with larger quantities of material the globulin antigen still possessed greater specificity. (Table 3.)

CONCLUSIONS

A method is described by which specific antigenic protein material may be obtained from mycelial masses of *Fusarium lini*. A fraction corresponding to globulin possesses antigenic specificity in a higher degree than prepared mycelial masses. It is suggested that in proportion as the method fails to extract globulin from the mycelial mass the mycelial mass retains antigenic specificity. Further refinement of the technic of obtaining globulin from a mycelial mass of *F. lini* is necessary before it will be possible to check the interrelationships of the physiologic strains by serological methods.

LITERATURE CITED

(1) BROADFOOT, W. C.
1926. STUDIES ON PARASITISM OF FUSARIUM LINI BOLLEY. Phytopatholgy 16: 951–978, illus.
(2) CHIBNALL, A. C.
1924. SPINACIN, A NEW PROTEIN FROM SPINACH LEAVES. Jour. Biol. Chem. 61: 303–308.
(3) ——— and NOLAN, L. S.
1924. A PROTEIN FROM THE LEAVES OF THE ALFALFA PLANT. Jour. Biol. Chem. 62: 173–178.
1924. A PROTEIN FROM THE LEAVES OF ZEA MAYS. Jour. Biol. Chem. 62: 179–181.
(5) DWORAK, M.
1926. THE EFFECT OF MOSAIC ON THE GLOBULIN OF POTATO. Jour. Infect. Diseases, 41: [215]–221, illus.
(6) HECK, A. F.
1929. A STUDY OF THE NATURE OF THE NITROGENOUS COMPOUNDS IN FUNGUS TISSUE AND THEIR DECOMPOSITION IN THE SOIL. Soil Sci. 27: 1–46, illus.
(7) NELSON, C. I., and BIRKELAND, J. M.
1929. A SEROLOGICAL RANKING OF SOME WHEAT HYBRIDS AS AN AID IN SELECTING FOR CERTAIN GENETIC CHARACTERS. Jour. Agr. Research 38: 169–181, illus.
(8) ——— and DWORAK, M.
1926. STUDIES ON THE NATURE OF WILT RESISTANCE IN FLAX. N. Dak. Expt. Sta. Bul. 202, 30 p., illus.

U. S. GOVERNMENT PRINTING OFFICE: 1933